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Neutrophil Clearance: when the party's over, cleanup begins

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Abstract

Maintenance of circulating, functional neutrophils and their robust recruitment to tissues in response to injury and/or microbial infection are critical for host defense. Equally important, though less well understood, are the processes for removal of these short-lived cells. Here we review recent findings of novel neutrophil characteristics that determine removal. These neutrophil-derived signals, in turn, can shape the responses of other cells and surrounding tissues and promote a return to homeostasis. If not removed, dying neutrophils disintegrate and release phlogistic cargo that can further contribute to ongoing inflammation, tissue destruction, or autoimmunity.

Removal of neutrophils from circulation and following tissue deployment

Neutrophils, the most numerous of circulating leukocytes, are readily mobilized innate immune cells that are critical for defense against bacteria and fungal pathogens [1]. These terminally differentiated cells are short-lived; their lifespans are generally measured in terms of hours, though a recent and provocative report suggests a circulation time in the blood of several days [2–4]. Neutrophils are continuously replenished during homeostasis, and their provision is augmented during infection or significant tissue injury, such that much of the bone marrow may become devoted to their production. Homeostatic removal of neutrophils from the circulation must match production and is mediated by macrophages in the liver, bone marrow stroma, and marginal zone of the spleen [4,5]. The kinetics of neutrophil production, release, equilibrium between circulating and margined pools, and their removal have been recently reviewed [3,4,6,7].

Following injury, with or without infection, neutrophils are recruited to tissues by chemotactic factors presented in a temporally and spatially defined manner [8,9]. Within tissues, the neutrophils orchestrate many mononuclear cell activities, and progressively unleash an arsenal of diverse constituents for host defense, *e.g.* reactive oxygen species (ROS), antimicrobial peptides and serine proteases, many with potential for inflicting further tissue injury [1,10,11]. These recruited neutrophils are largely removed *in situ* by macrophages, although to a small extent also by dendritic cells and/or exodus to draining lymph nodes and following migration outside the body as in airways, gut, gingival crevice, etc. [12]. Despite their large numbers relative to tissue macrophages, neutrophil

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disappearance in resolving inflammation is often dramatic, such that detection of morphologically dead neutrophils is relatively infrequent in tissues during resolution (Fig. 1). In this context, it is important to recognize that neutrophil numbers at any given time represent simultaneous recruitment and removal combined, and few studies address the absolute flux of these cells within a given lesion. Thus, as we will discuss, in the absence of defects in clearance, removal is an efficient, high capacity process that in fact actively promotes resolution and restoration of tissue structure and function.

What marks a neutrophil for clearance? The focus of this review is to highlight emerging data regarding different mechanisms for neutrophil demise, their repertoire of signals to engage the macrophages, and to some extent, the consequences of these interactions. We start with three underlying concepts: i) neutrophil removal from either the circulating pool, or from tissues, depends on signals resulting from neutrophil death, activation or aging (referred to here as neutrophil “fates”), rather than by stochastic processes; ii) different neutrophil “fates” have unique phenotypic signatures, but also share an overlapping repertoire of signals that are important for recognition; and iii) the consequences of recognition and removal, or its failure, depend on the “mix” of neutrophil-derived signals both quantitatively and qualitatively. Two caveats also deserve mention: much of the data come from *in vitro* studies and murine models, hence, extrapolating their relevance in human health and disease is often speculative.

Neutrophil “fates”: dead, activated or aged

Cell death is an irreversible endpoint, but lacking a precise definition, may be considered to have occurred when i) plasma membrane integrity is lost, ii) the cell fragments into discrete bodies, or iii) the cell is engulfed by an adjacent cell [13,14]. While neutrophil longevity during inflammation may be extended by various stimuli including pattern recognition signals, growth factors or chemokines, it can also be shortened following phagocytosis of a number of pathogens (reviewed in [2,7]). Ultimately, however, neutrophils succumb via an expanding repertoire of possible death pathways (Fig. 2 and text box). Most importantly, these all result in the expression and/or exposure of signaling and recognition structures that can, under normal conditions, promote removal of the dying cells.

Conceptually, neutrophils may also signal for removal before actually dying. Recent investigation has found that brief activation of the neutrophil NADPH oxidase generates surface ligands similar to those on apoptotic neutrophils, that enhance removal both *in vitro* and *in vivo* [15,16] (see below). These findings are consistent with an older report (in need of reinvestigation) that over-expression of the pro-survival protein, Bcl-2, to inhibit neutrophil apoptosis, nevertheless, did not delay their normal clearance [17]. Similarly, aging of neutrophils in the circulation may generate signals prior to obvious apoptosis/cell death. Murine studies that identify a senescent neutrophil phenotype implicate CXCR4 re-expression in neutrophil removal in the bone marrow [4,6]. Whether such processes apply to the bulk removal of the cells in the liver and spleen is unknown. However, mathematical modeling in man suggests that neutrophil clearance, is not exclusively senescence-based, supporting the possible role of signals such as those associated with activation and/or apoptosis.

Although death endpoints are depicted as discrete in Fig. 2, overlap is considerable (text box). For example, neutrophils ingesting community-associated methicillin-resistant *Staphylococcus aureus* or *Streptococcus pyogenes* can undergo necrosis or accelerated apoptosis which culminates in cell lysis, so-called secondary necrosis [2,18]. Further, as outlined above, the underlying mechanisms driving death processes, often overlap. For example ROS from the NADPH oxidase may be essential for NETosis and autophagy, and

often play a predominant role in apoptosis [19,20]. The latter is especially evident following pathogen ingestion [2], and the lack of ROS can prolong survival of neutrophils from chronic granulomatous disease patients [21]. ROS from various sources are also heightened in necrotic tissue. Hence the signals deriving from these different forms of neutrophil “death” are also significantly overlapping.

Neutrophil death: several ways to go

Cellular **necrosis** follows an insult so devastating that influx of extracellular ions is rapid, and causes cell and organelle swelling, permeabilization of the plasma membrane and release of intracellular constituents.

Programmed cell death on the other hand is a genetically determined orchestrated death, and several processes have been described in neutrophils:

Neutrophil **apoptosis** can be mediated via both extrinsic and intrinsic pathways, and roles for ROS from the NADPH oxidase and/or mitochondria, increased calcium flux, activation of caspases, cathepsins and calpains, and pro-apoptotic proteins of the Bcl-2 family have been variously demonstrated [2,7]. Apoptosis is generally recognized by characteristic chromatin fragmentation, chromatin and cytoplasmic condensation with the formation of small apoptotic bodies. However, while neutrophils do exhibit the nuclear changes and undergo cell shrinkage, they do not release blebs or fragment to the extent of many cells. They do shed receptors and become functionally effete, demonstrating impaired chemotaxis, degranulation and ROS generation (reviewed in [2]).

A relatively newly described form of programmed cell death is called **NETosis**, for the striking generation of neutrophil extracellular traps (NETs) from decondensed nuclear chromatin. The process involves NADPH oxidase-dependent dissolution of the nucleus and intracellular membranes and rupture of the plasma membrane [20]. Notably, viable, intact neutrophils can also produce NETs depending on the conditions of stimulation; in this case, by ROS-dependent extrusion of mitochondrial DNA [22]. Thus, the appearance of NETs does not necessarily signify neutrophil death. NETs are thought to both physically contain microbes and concentrate neutrophil antimicrobial products for enhanced killing and has been demonstrated in a number of *in vivo* systems [20,23].

Neutrophils also undergoing **autophagy-associated death** have been recently recognized. Autophagy is a recycling process prompted by conditions of nutrient limitation, in which a cell ingests and degrades redundant or damaged organelles and macromolecular components into double- or single-membrane lysosomal-derived vesicles. Large vacuoles in neutrophils associated with infection and autoimmunity likely represent autophagosomes [24]. *In vitro*, such structures can be identified following stimulation, in some cases in the presence of pro-survival factors such as GM-CSF to inhibit caspase activation and apoptosis [24]. These data suggest potential branch points in death pathways in which autophagy represents a survival pathway but when it fails, leads to apoptosis [25]. In neutrophils, however, these pathways have not been clearly delineated, though a requirement for a functioning NADPH oxidase has been demonstrated in autophagy [19].

Although a number of other types of programmed cell death have been described, *e.g.* pyroptosis, and oncosis [2,23], it is not clear at this point how relevant they are to neutrophil death processes.

An ever-expanding repertoire of signals are released from, or exposed on the surface of dying (or activated) neutrophils. These are then recognized by macrophages and other cells of the inflammatory milieu, and can lead to removal of the neutrophils as well as both anti-

and pro-inflammatory consequences. Many of the intracellular constituents released during neutrophil necrosis are recognized by macrophages via TLRs and NLRs as damage-associated molecular patterns (DAMPs), *e.g.* cationic proteins, uric acid, HMGB-1, chromatin-bound IL-1 α , etc. [26,27]. Typically such signals result in activation of NF κ B resulting in pro-inflammatory cytokine production. Released proteases, such as neutrophil elastase can also be proinflammatory [18]. However, other neutrophil intracellular proteins such as annexin 1, α -defensin, and S100A9 are anti-inflammatory and may modulate the effects ordinarily associated with the recognition of DAMPs [28–30].

By striking contrast, apoptotic cells, including neutrophils, are more generally anti-inflammatory, anti-immunogenic and tissue restorative in that their recognition by macrophages and other cells can stimulate to various degrees production and secretion of factors such as TGF β , IL-10, VEGF, etc. [31–33]. Notably, the plasma membrane of apoptotic cells remains intact initially. With time, if removal of the apoptotic cell is delayed, plasma integrity is lost (secondary necrosis, Fig. 2) but it is noteworthy that the apoptotic neutrophil seems to maintain its intact membrane for longer than most cells, possibly because of its reliance on glycolysis as an ATP source [12,34]. This persistence as an intact cell may importantly allow time for complete removal of a cell type that is so richly endowed with potentially tissue-damaging constituents. Further, *in vitro*, and most certainly *in vivo*, death and dying of a population of neutrophils is neither synchronous, nor homogeneous. For example, following optimized activation *in vitro*, only approximately a third of neutrophils can be induced to undergo death by NETosis [23].

“Palatability” of neutrophils, and their efferocytosis by macrophages

The process of cell corpse removal, called efferocytosis (“*to carry to the grave*”) has been best studied with regard to the signals and mechanics involved in the removal of apoptotic cells, including apoptotic neutrophils. Colloquially, these signals have been designated as “find me” signals that attract macrophages to the site of cellular demise, and “eat me” signals on the apoptotic cell surface that often cluster into signaling platforms (Table 1, Fig. 3) [12,35]. Of known “eat me” signals, many are bound “bridge” molecules, soluble pattern-recognition proteins of the innate immune system present in the milieu that opsonize the apoptotic target cell (reviewed in [36]). A number of changes on the dying cell surface lead either to binding of such molecules or to direct engagement of macrophage receptors. These include externalized phosphatidylserine (PS), altered lipids, changes in surface charges likely attributed to amino sugars, and exposure of intracellular molecules such as calreticulin, mitochondrial and nuclear constituents (Table 1). Amongst these signals, some ligand combinations promote adherence or “tethering” to macrophages, while others “tickle” the macrophage to induce uptake, or others do both, in a two-step “tether and tickle” model (Fig. 3) [12]. Further, these effects are opposed by “don’t eat me” systems, repulsive signals found on viable cells that must be removed, sequestered or otherwise disabled for uptake to occur.

The engulfment process, under the control of the RhoGTPases, Rac1 and Cdc42, is thought to result in the formation of a spacious phagosome surrounding the apoptotic cell and imbibed fluid from the extracellular milieu by a process akin to macropinocytosis [12]. Others have suggested that the macrophage membrane surrounds the apoptotic cell by a “zipper” mechanism where ligands on the apoptotic cell are sequentially engaged to form a tight-fitting phagosome, at least with cells early in the apoptotic process [37]. The overall processes almost certainly depend on the state of the cell and the combined repertoire and presentation of signals. For instance, necrotic neutrophils may release intracellular constituents acting as “find me” signals, but have little chance to assemble “eat me” signaling platforms or disable “don’t eat me” signals. NETosing neutrophils may be

recognized by exposed DNA and histones, but their efferocytosis may be slowed by PR3 (Table 1; see below) and degradation of the NETs by DNases may be required for their removal. Further descriptions of individual molecules, particularly where information is available to implicate roles in the removal of dead, activated or aged neutrophils, are described in greater detail below and shown in Table 1.

“Find me” signals

A number of diffusible signals, *e.g.* ATP and UTP, and DAMPS released from permeabilized cells and to some extent apoptotic cells [35,38], are chemoattractants for many types of inflammatory cells. Other “find me” signals released from apoptosing cells are more selective for monocytes/macrophages and dendritic cells (*e.g.* fractalkine, lysophosphatidylcholine, and sphingosine-1-phosphate) [35,38], and others, such as lactoferrin, appear to actively inhibit neutrophil migration [39]. The roles of such “find me” signals in orchestrating neutrophil removal under various inflammatory circumstances have not been thoroughly investigated, and it might be argued that such signals are unnecessary: monocytes and macrophages are invariably recruited in acute inflammation by monokines and products released by activated neutrophils or other inflammatory cells, *e.g.* eicosanoids and alarmins such as α -defensin [10,40], as well as products of inflammatory tissue injury. Notably, although the lipid signal, lysophosphatidylcholine has been described as a “find me” signal for late apoptotic cells of various cell types, we have been unable to identify this lipid product in apoptosing neutrophils after prolonged culture [15].

“Eat me” signals

The best described “eat me” signal is phosphatidylserine, PS. Normally sequestered in the plasma membrane inner leaflet, PS exposure on the cell surface leads to recognition of its headgroup by an increasing number of bridge molecules and macrophage receptors (Table 1) [12,36]. The near-universal appearance of PS on apoptotic cells, and its detection by annexin V, has become a standard assay for detection of apoptosis. However, PS is also exposed, at least to some degree, as neutrophils (and other cell types) undergo activation [16,41] and by definition, after membrane disruption as in necrosis, and death associated with NETosis [42]. PS exposure, even in the absence of evidence of apoptosis by TUNEL staining, was shown to mediate neutrophil uptake into the liver of rats following LPS injection [4]. Maintenance of PS on the inner leaflet of unstimulated, viable cells requires ATP and aminophospholipid translocases. However, translocase function is impaired by oxidative or nitrosative stress, ATP depletion or high levels of intracellular calcium during cell death [12]. Also, as a separate event, increased transbilayer flip-flop of all phospholipid classes (which leads to PS exposure) occurs during neutrophil activation as well as apoptosis. Whether phospholipid flip-flop represents a membrane repair process or is the result of specific “scramblases” or “floppases” is not yet clear [43,44]. Calreticulin is an additional “eat me” signal recognized by LDL receptor related protein (LRP) on macrophages or by collectins as bridge molecules [45]. A number of additional bridge molecules have been associated with neutrophil removal. These include thrombospondin, annexin 1, deposited complement, and long pentraxin (PTX3) stored in neutrophils and translocated to the surface during apoptosis [2,12,30,46].

Oxidized phospholipids (oxPL), of both the PS and phosphatidylcholine (PC) subclasses, are generated in some dying cells, and enhance binding of certain bridge molecules to facilitate recognition through scavenger receptors (*e.g.* CD36) expressed on macrophages [47]. Considering the oxidizing capacity of the neutrophil NADPH oxidase, it was hypothesized that oxPL species would comprise “eat me” signals during both activation and apoptosis. Surprisingly, oxPL species were not produced by human neutrophils *in vitro* or murine neutrophils *in vivo* in a peritonitis model [15,16]. In contrast, up to 20% of the PS pool was

converted to a PS species containing only one fatty acyl constituent, lysophosphatidylserine (lyso-PS) in an NADPH oxidase-dependent manner. Lyso-PS remained cell-associated and signaled to macrophages via the G-protein receptor, G2A, to enhance, but not initiate, neutrophil removal *in vitro* and *in vivo* [15,16]. As such, lyso-PS may be a key signal for high capacity uptake of activated neutrophils even before they show signs of programmed cell death.

“Don’t eat me” signals and suppressors of efferocytosis

A number of molecules have been identified as “don’t eat me” signals on viable neutrophils. CD47 on viable cells signals to macrophages via the inhibitory receptor, SIRP α , and such signaling is diminished with apoptosis leading to enhanced uptake [45,48]. Similarly, PAI-1, a member of the SERPIN family of serine protease inhibitors, appears to colocalize with calreticulin on viable neutrophils, where it is thought to block or diminish its “tickling” signal to macrophages, but it segregates from calreticulin during neutrophil apoptosis [49]. Genetic deficiency or blockade of PAI-1 rendered both viable and apoptotic neutrophils more palatable to macrophages, an effect abrogated by its replenishment. Other candidates include the urokinase receptor (uPAR) and CD31 both of which have altered expression during neutrophil activation and/or apoptosis [50–52].

Suppressors of neutrophil removal are also described, *e.g.* PR3, which is mobilized from neutrophil secretory vesicles to the cell surface along with PS during early apoptosis [53]. Following genetic deletion of PR3, efferocytosis of neutrophils doubled *in vitro*. Though enzymatic activity of PR3 was not required, hydrophobic interactions of PR3 to signaling lipids was hypothesized. Likewise, released neutrophil elastase and cathepsins may serve to limit efferocytosis by cleaving macrophage surface receptors or ligands on the surface of the apoptosing cell, leading to inefficient clearance as seen, for example, in cystic fibrosis [54].

The milieu and macrophage programming in efferocytosis of neutrophils

Aside from signals on the neutrophil itself, efferocytosis obviously depends on factors in the microenvironment and the status of macrophage programming which determines their “readiness” to engulf [55–57]. First, availability of bridge molecules is critical. For example, efferocytosis is much more efficient in the presence of serum *in vitro*, and presumably proteins in the interstitial fluid and inflammatory exudate contribute *in vivo*. Notably, some bridge molecules are tissue specific, *e.g.* SPA and SPD are found primarily (though not exclusively) in the lung, and other bridge molecules such as MFG-E8 are produced by macrophages themselves. Genetic manipulation has demonstrated significant roles for individual bridge molecules (*e.g.* MFG-E8 and C1q [12]). Other efferocytosis enhancers in the milieu have also been described. Annexin 1 and derived peptides (released by activated and apoptosing neutrophils themselves as well as other cells [30,58]), adiponectin, and the lipids, lipoxins, protectins, resolvins, and maresins, enhance efferocytose or general phagocytic capability [59,60]. In the opposite direction, inhibitors such as TNF α and oxidants likely add to the balance that controls overall efficiency of neutrophil removal [12,61].

Macrophages programmed towards so-called “alternative activation” are more efficient at efferocytosis. Pivotal roles for the nuclear receptors, LXR, PPAR γ and/or PPAR δ , which in turn, upregulate efferocytic receptors and bridge molecules, have been demonstrated [55,57,62]. Interestingly, PS itself can drive PPAR γ upregulation [63] as can oxidation products which act either as PPAR γ activators or via Nrf2 [64], suggesting that apoptotic cells in general, and neutrophils in particular, might influence their own uptake by macrophages. However, under certain circumstances, exposure to apoptotic cells can also lead to macrophage deactivation via adenylyl cyclase activation leading to their inability to

efferocytosis [65]. Conversely, programming of macrophages towards “classical activation” is associated with decreased efferocytosis, and its association with a growing list of chronic autoimmune states disease states, for example, SLE, COPD, CF, severe asthma and CGD [31,54,66,67], raises intriguing questions about the role in these circumstances for defective neutrophil clearance. Notably, macrophage programming can be modulated therapeutically. For example glucocorticoids, statins, azithromycin all enhance efferocytosis [54,58,68], and therapeutic IFN γ nonspecifically enhances general phagocytic capacity [69].

Consequences of apoptotic neutrophil recognition

The consequences of neutrophil recognition and removal depend on neutrophil fate, the signals generated, programming of macrophages, and the playing field in which these events take place. Given the size of this subject, only a few outcomes are highlighted below and the reader is directed to several recent references [18,31,32,54,67,70,71].

Physical removal of neutrophils is the most obvious consequence of recognition by macrophages, and is required to return tissues to their normal architecture and function. While it is thought that neutrophils and their debris are ultimately digested within the macrophage phagolysosome, macrophages have also been shown to co-opt ingested neutrophil granule proteins for antimicrobial defense, *e.g.* against mycobacteria [72]. Removal also serves to protect tissues from further injury due to neutrophil constituents. Conversely, failure of removal, allows further deterioration of neutrophils regardless of mechanism of death [18]. If enough disintegrate in a given locale, tissue liquefaction results in formation of a puss-filled abscess [1]. In other instances, failure of neutrophil removal results in the so-called “nuclear dust” characteristic of lesions of ANCA positive polyarteritis [73]. Significantly, autoimmunity is often associated with clearance defects, and in particular, the production of anti-neutrophil antibodies [18,67]. Specifically the generation of ANCA directed against neutrophil PR3 and other cytoplasmic epitopes are both a marker of defects in clearance and are pathogenic by heightening neutrophil death and their inflammatory recognition [74].

A major role of macrophages is the production of molecules, *e.g.* cytokines and chemokines that “shape” inflammatory, as well as homeostatic tissue events [5,75]. The recognition of dying cells, results in profound alterations in macrophage responses. As noted above, responses to neutrophils, dying, activated or aged, represent a continuum (Fig. 2). At one extreme, TGF β and/or IL-10 production by macrophages exposed to apoptosing neutrophils suppresses pro-inflammatory cytokine, chemokine and eicosanoid production (Fig. 2). While anti-inflammatory signaling is thought of as important for resolution of inflammation, the consequences are not always without detriment. Over-exuberant fibrotic responses driven by TGF β are an example. Another is the immunosuppression of macrophages that accompanies ingestion of infected neutrophils, the so-called Trojan horse effect, which results in enhanced infection by certain microbes [12]. Under homeostatic conditions, neutrophil recruitment into tissues and their recognition by phagocytes is thought to provide a “sensor” for regulating a neutrophil “turnstile,” matching bone marrow production and release with removal [5,6,76]. Interestingly, depending on the model, divergent macrophage “sensor” populations appear to respond differently with regard to production of G-CSF, a growth factor essential for neutrophil production and release from the bone marrow. On the one hand, recognition and engulfment of senescent neutrophils by bone marrow stromal macrophages reportedly enhances G-CSF production [6]. Alternatively, recognition of neutrophils by peripheral tissue macrophages reportedly suppresses production of G-CSF to “tune down” neutrophil production [5,76]. In these latter models, blockade of neutrophil migration into tissues and/or their recognition by macrophages resulted in heightened G-CSF-driven neutrophil production. In other models, phagocyte recognition of neutrophils

containing infected material enhances their production of TGF β , but also of IL-6 and IL-23, which together generate Th17 cells that subsequently enhance G-CSF for “stress” neutrophil production [70]. The resultant neutrophilia insures reinforcements for sterilizing tissues. As hypothesized, the signals produced on or by neutrophils, aged, activated or dying, and the character of pertinent factors in the milieu, including the programming of the responding macrophages, are likely key in determining the varying outcomes of their interaction.

Concluding remarks

Undeniably, the primary function of neutrophils is host defense that, in turn, depends on adequate numbers in circulation and timely recruitment to sites of bacterial and fungal invasion. Once sterilization is accomplished, removal of neutrophils is imperative for the return of tissues to their functional state and to prevent further injury as these short-lived cells die and lose integrity. Aside from antimicrobial duties, neutrophils are also recruited during acute inflammation (*e.g.* in ischemia-reperfusion) and various chronic disorders (*e.g.* chronic obstructive pulmonary disease), where they contribute to tissue injury and underlying pathology. Emerging data provide new insights into the expanding array of mechanisms by which these cells are recognized and removed by phagocytes. The repertoire of signals generated by and on neutrophils as they age, are activated or ultimately die, either in the circulation or on the battlefield of injured or infected tissues, helps to titrate their numbers, modulate their activities and determine their legacy. Though much remains to be learned, an increasingly sophisticated understanding of the nature of these signals, their interpretation by other cells within the tissues, and their roles in the orchestration of subsequent host responses, may allow us unique opportunities to intervene and modify such responses where needed to either enhance host defense, or conversely, prevent inappropriate injury.

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Abbreviations

ANCA	anti-neutrophil cytoplasmic antibodies
ATP	adenosine triphosphate
BAI1	brain-specific angiogenesis inhibitor 1
CF	cystic fibrosis
CGD	chronic granulomatous disease
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
Del-1	developmental endothelial locus-1
FcR	Fc receptor
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte macrophage-colony stimulating factor
HMGB-1	high-mobility group protein B1
IL-6	interleukin 6

IL-10	interleukin 10
IL-23	interleukin 23
IFNγ	interferon gamma
LDL	low-density lipoprotein
LOX-1	oxidized LDL receptor 1
LPS	lipopolysaccharide
LRP	lipoprotein receptor-related protein
LXR	liver X receptor
Lyso-PS	lysophosphatidylserine
MBL	mannose binding lectin
MFG-E8	milk fat globule-EGF factor 8 (lactadherin)
MMR	macrophage mannose receptor
NADPH	nicotinamide adenine dinucleotide phosphate
NETs	Neutrophil Extracellular Traps
NFκB	nuclear factor kappa B
NLR	Nod-like receptor
Nrf2	NF-E2-related factor 2
PAFR	platelet activating factor receptor
PAI-1	plasminogen activator inhibitor-1
PC	phosphatidylcholine
PPARδ	peroxisome proliferator-activated receptor delta
PPARγ	peroxisome proliferator-activated receptor gamma
PR3	proteinase 3
PS	phosphatidylserine
PTX3	pentraxin-related protein 3
ROS	reactive oxygen species
SERPIN	serine proteinase inhibitors
SIRPα	signal-regulatory protein alpha
SLE	systemic lupus erythematosus
SPA	surfactant protein A
SPD	surfactant protein D
SRA	scavenger receptor A
SRB	scavenger receptor D
TAM	Tyrosine Kinase Mer
TGFβ	transforming growth factor beta
Th17	T helper 17

TIM	T cell Ig and mucin
TNFα	tumor necrosis factor alpha
TLR	Toll-like receptor
TUNEL	terminal deoxynucleotide transferase dUTP nick end labeling
uPAR	urokinase plasminogen activator receptor
UTP	uridine triphosphate
VEGF	vascular endothelial growth factor

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Fig. 1. Time course of tissue neutrophilia in acute inflammation

a). Following tissue injury or infection (*e.g.* zymosan-induced peritonitis), neutrophils (N) are simultaneously recruited to the site and removed *in situ* by macrophages (M). Neutrophilia often resolves dramatically with accumulation of few apoptotic neutrophils supporting mechanisms for efficient, high-capacity clearance. b) Time course of efferocytosis by macrophages in peritonitis.

Fig. 2. Possible death pathways for neutrophils and impact on resolution or perpetuation of inflammation

Recruited neutrophils succumb to various death pathways within tissues (blue boxes). Based on signaling for their recognition by macrophages, and the stage at which they are removed, inflammatory consequences will vary across a continuum from anti-inflammatory and immunosuppressive to pro-inflammatory and immunogenic.

Fig. 3. Signaling for efferocytosis

An apoptotic cell presents signals on its surface or recruits bridge molecules from the milieu that serve as “eat me” signals to macrophages. “Eat me” signals both tether the apoptotic cell to the macrophage and “tickle” or signal for it to reorganize its cytoskeleton for uptake. Uptake is opposed by “don’t eat me” signals usually cleared from the cell or segregated from “eat me” signals as the neutrophil undergoes apoptosis. Other factors serve as enhancers to improve efficiency of uptake, or as inhibitors to suppress uptake. *Figure published with permission of the Journal of Leukocyte Biology.*

Table 1Ligands, bridge molecules, and receptors for the recognition of dying cells^a

Ligand on dying cell	Bridge molecules	Macrophage receptor
“Eat me” signals		
PS ^b		PS recognition structures (BAI1, TIM-4, TIM-1, TIM-3, Stablin-2); scavenger receptors (SRA, SRB, etc.)
“	Annexins 1 and 2	Homophilic interaction
“	C1q	C1q receptors; Calreticulin/LRP (CD91)
“	β2 glycoprotein 1	?
PS; oxidized PS ^c	Gas6, Protein S	TAM Rs (Tyro, Axl, Mer)
“	MFG-E8, Del-1	αvβ3/β5 integrins
“	Collectins (SPA, SPD)	LRP (CD91)
“		CD36
Oxidized PC ^c		CD36; other scavenger receptors; LOX-1; CD14; TLR4; PAFR
Oxidized PC ^c ; phosphorylcholine	Pentraxins (<i>e.g.</i> CRP); IgM natural antibodies; deposited complement after activation;	Complement Rs (CR3, CR4, CR1);
	IgG autoantibodies	FcR
Cardiolipin; oxidized cardiolipin	IgM natural antibodies; deposited complement after activation	Complement Rs
Calreticulin ^b		LRP (CD91)
Mitochondrial and nuclear nucleic acids and associated constituents (histones, <i>etc.</i>)	Collectins, C1q, pentraxins (<i>e.g.</i> CRP), C4b binding protein, properdin, deposited complement after activation	LRP (CD91); complement Rs
Altered carbohydrates, nucleic acids, phospholipids	Ficolins; MBL; deposited complement after activation	MMR; lectin receptors; LRP; complement Rs
C3b and iC3b deposition		CR3, CR4, CR1
CD31 ^c	fibronectin	αvβ1/CD31
CD43, polyglycosamine side chains		Nucleolin
CD14 ^c		ICAM3
PS; unknown	Thrombospondin ^b	CD36/αvβ3/β5; LRP
?	adiponectin	Calreticulin/LRP
--	Crosslinking antibody	CD44 ^b
PTX3 translocated to the surface of late apoptotic neutrophils	C1q and deposited complement after activation	LRP (CD91); complement Rs
“Don’t eat me” signals		
CD47 ^b		SIRPα
CD31		CD31 (homophilic interaction)
PAI-1 ^b		
uPAR ^b		uPAR (homophilic interaction)

Ligand on dying cell	Bridge molecules	Macrophage receptor
PR3 ^b		?
Nuclear debris	Soluble PTX3	?

^a Refer to text above and to references [12,35–36] and references therein.

^b Specifically shown to play a role in neutrophil removal.

^c Demonstrated for other cell types, and studied in neutrophil removal, but role in neutrophil removal not demonstrated.

Abbreviations: PS, phosphatidylserine; BAI1, brain-specific angiogenesis inhibitor 1; TIM, T cell Ig and mucin; SRA, scavenger receptor A; SRB, scavenger receptor D; LRP, lipoprotein receptor-related protein; TAM, Tyro Axl Mer; MFG-E8, milk fat globule-EGF factor 8 (lactadherin); Del-1, developmental endothelial locus-1; SPA, surfactant protein A; SPD, surfactant protein D; PC, phosphatidylcholine; LOX-1, oxidized LDL receptor 1; PAFR, platelet activating factor receptor; TLR, Toll-like receptor; CRP, C-reactive protein; MBL, mannose binding lectin; MMR, macrophage mannose receptor; PTX3, pentraxin-related protein 3; SIRPα, signal-regulatory protein alpha; PAI-1, plasminogen activator inhibitor-1; uPAR, urokinase plasminogen activator receptor; PR3, proteinase 3.