Unexpected Role of Non-Immune Cells: Amateur Phagocytes

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Abstract

In the physiological state, cellular debris is generated in different vesicles forms such as exosome, microvesicle/microparticle, and apoptotic body for communication purposes and maintaining body homeostasis. Under pathological state, apoptotic bodies are predominantly secreted abundantly by unhealthy cells which are recognized and eliminated by professional phagocytes (PPs). However, accumulating evidence suggests a novel key role for amateur phagocytes (APs) to identify and remove cellular debris located in different tissues and organs, presumably before PPs reach the injured area. In this review, we present diversity of APs in mammals and examine different mechanism of cellular debris engulfment and clearance. Finally, we discuss the implications of apoptotic body incorporation on cytokine release, involvement of cellular organelles to eliminate cellular debris, and cellular reprogramming phenomena. A comprehensive understanding of the role of APs is necessary to enable effective intervention strategies for the prevention and treatment of many diseases.

Keywords Amateur phagocytes. Apoptotic body. Autophagy. Cellular debris. Efferocytosis.

Introduction

Billions of cells die within our body on a daily basis due to natural processes, disease, or microorganism evasion. Excessive cellular debris emerges through various cell death programs which yield different responses to other cells and the cell environment. Unresolved cellular debris causes progression of a number of diseases such as Alzheimer's

and Huntington's disease [1]. Cellular debris can not only be harmful, but it is also required to maintain stem cell homeostasis, ameliorate osteopenia [2] and contribute to the removal of non-perfused vessel segments and reduction of endothelial cells density during vessel maturation in the retinal development[3]. Generally, cell debris engulfment is performed by professional phagocytes such as monocytes and neutrophils, however a number of studies suggest that other cells, known as non-professional phagocytes or amateur phagocytes (APs) or neighboring cells, are also capable of performing phagocytosis. APs can be observed throughout human body and exert phagocytic activity only when needed, especially under disease, stress, or pathogen invasion. For instance, microvascular endothelial cells (MVECs) in the human brain are involved in myelin debris elimination generated from breakdown of myelin sheaths after spinal cord injury (SCI) via opsonization recognition and autophagy clearance mechanism [4]. Moreover, myofibroblasts, which execute tissue fibrosis by producing extracellular matrix protein, have also been implicated in engulfing dead cells in myocardial infraction mediated by milk fat globule-epidermal growth factor 8 (MFG-E8) [5]. These suggest that under certain circumstances, APs turn on different mechanisms to engulf and remove cellular debris. In addition, post-degradation processes show exciting phenomena to be explored, such as competition of immune responses (pro vs anti-inflammatory cytokine elicitation) and cellular reprogramming phenomena. In this review, we present recent advances in our understanding of APs and particularly focus on how cell debris signaling induces APs engulfment and clearance and their effects on APs themselves and their surroundings.

Cell debris generation

Cell death is an important process in the body as it promotes the removal of unwanted cells. There are three major cell death programs which have distinct features: apoptosis, necrosis, and autophagy [6]. For decades, apoptosis and necrosis classification has been heavily based on their membrane plasma morphology. However, factors affecting membrane integrity might be induced by heterogeneous insults and shared morphological characteristics by late apoptosis and necrosis might create biased results. Thus, the Nomenclature Committee on Cell Death (NCCD) recommends using biochemical markers to distinguish these mechanisms [7]. Hou et al. [8] observed chromatin detachment and nuclear envelope collapse mediated by phosphorylated barrier-to-autointegration factor (BAF) in necrosis, whereas chromatin and nuclear envelope remained intact in apoptosis. Unlike apoptosis and necrosis, autophagy can be identified through a cellular component formation, namely the autophagosome [9].

Of note, autophagy is also a pro-survival mechanism, and thus leads to the highest survival rate in all cell death types, followed by apoptosis and necrosis. Ideally, a cell would "*choose*" autophagy over apoptosis, and apoptosis rather than necrosis if autophagy is impeded. Furthermore, these mechanisms are interpreted and can be activated concomitantly in response to stimuli. Necroptosis is a form of regulated necrotic cell death that shares similar pathways with apoptosis, i.e. inhibition of caspase-8 (apoptosis) activates receptor interacting protein kinase 1/3 (RIP1/3) complex (necrosis) through phosphorylation and mediates necroptosis [9]. Despite the three principal cell death mechanisms mentioned above, a variety of modalities of cell death have been discovered over the past decades and are discussed comprehensively in Galluzzi et al [7].

Unlike exosomes and microparticles which are secreted by healthy cells, apoptotic bodies are the result of unhealthy cells disassembly. Once cells commit suicide through a specific cell death pathway (except necrosis), fragmented cells release mixed molecules which are associated with membrane vesicles or apoptotic bodies [10]. Apoptotic bodies possess two subtypes, large membrane-bound vesicles and smaller apoptotic microparticles. In particular, cell-derived microparticles are defined as 0.1-1 μm subcellular membrane vesicles that arise during cell activation or apoptosis with a lack of nucleus or synthetic capacity: these may contain cytoskeletal protein, and have some quantity of phosphatidylserine [11]. Their composition and functional properties vary with their cellular origin and the type of stimulus involved in their formation. Along with microparticles, apoptotic process cells also release a large number of 1–5 μm extracellular vesicles named apoptotic bodies that can be identified and engulfed by phagocytes for clearance purpose [12].

A high rate of cell death is an inevitable consequence of impaired cell or tissue function due to external stimuli (e.g. environmental pollutants, allergens and pathogens) [13] or internal stimuli (e.g. inflammatory signals [14] and autoimmune disease [15]). Pathogen elimination by professional phagocytes can induce cell death in the professional phagocyte itself e.g. bursting of neutrophils after *Leishmania major* engulfment, and then clearance of the dead phagocytes by other professional phagocytes, e.g. macrophages. Interestingly, specific pathogens can direct specific cell death fate, e.g. *Mycobacterium tuberculosis* infected-macrophages stimulates necrosis rather than apoptosis [16]. Furthermore, cells may undergo multiple cell death programs as reflected in atherosclerosis disease.

Excess low-density lipoprotein (LDL) build up along the artery is a sign of damage, and results in the sending of "eat me" signals to macrophages which in turn leads to foam cell formation. The activity of LDL clearance triggers the release of harmful inflammatory signals which cause the death of foam cells. The increased inflammation renders efferocytosis defective, resulting in secondary apoptosis and the promotion of a large amount of cell debris in the atherosclerotic plaque or necrotic core [17].

Diversity of Amateur Phagocytes (APs)

Numerous studies have documented and unveiled the existence of non-professional phagocytes, both in embryonic development and the adult stage across taxa. *C. elegans*, the most studied invertebrate organism in the context of understanding the role of non-professional phagocytes, has a variety of APs including hypodermal cells, gonadal sheath cells, pharyngeal muscle cells and endothelial cells [18]. However, here we only focus on APs diversity in mammals. In vertebrate embryogenesis, reactivated trophectoderm murine cells during morula-blastocyst formation can phagocytose adjacent uterine epithelium debris with a notable amount of multivesicular bodies (MVBs) [19]. Furthermore, stem cells function as non-professional phagocytes are described by mesenchymal cells in the macrophage-less PU.1 null mouse embryo acting to sculpt a webbed footplate and transform it into free interdigital space [20]. Furthermore, neural progenitor cells in adulthood can remove dying neurons [21]. Particularly in humans, APs manifest in different type of cells (Table 1) throughout the organs (brain [4,22], eyes [23], digestive tract [24,25], heart [5], lung [26,27], and kidney [28]). The location in which APs exist reflects dynamic cell activities promoted by mechanical, chemical, or biological stimuli and thus leads to rapid cell turnover. The inability of professional phagocytes to penetrate a certain location such as the blood brain barrier or the absence or delay of professional phagocytes' arrival in injury location emphasizes the indispensability of APs to assist or be a key player in eliminating dead cells and maintain homeostasis.

Considering the diversity and fundamental function of APs, it is tempting to propose a hypothesis that APs may initiate the cellular engulfment and clearance at the early and resolution stages of inflamed tissue. There are two reasons to support this statement: 1) when injury occurs, healthy neighbouring cells can sense it immediately rather than relying on chemokine release to recruit professional phagocytes [24] and 2) Professional phagocytes secrete

insulin growth factor 1 (IGF-1) and influence nearby APs by suppressing uptake of larger apoptotic cells and enhancing engulfment of smaller particles (150-200 nm) [29].

The Mechanism of Phagocytosis by Amateur Phagocytes (APs)

Phagocytosis is the process of recognition and ingestion of pathogens or cellular debris, whereas efferocytosis targets only apoptotic bodies. Phagocytosis and efferocytosis share similar pathways and can be generally categorized into 4 steps: 1) the release of 'find-me' signals by dying cells to recruit phagocytes, 2) phagocyte recognition and engagement of 'eat-me' signals on dying cells, 3) the engulfment of the cellular corpse, and 4) the processing, degradation, and immune response to the engulfed corpse [30].

There are various ligands (find-me) and receptors (eat-me) possessed by APs as illustrated in figure 1. One of the most-studied eat-me signals is phosphatidylserine (PS) which is distributed in the cytoplasmic leaflet of healthy cells and controlled by flippases, such as P4-ATPases. Nonetheless, dying cells or early apoptotic cells cause PS translocation to the cell surface and this flags the cell engulfment by inducing scramblase-dependent Ca^{2+} and caspase such as transmembrane protein 16F (TMEM16F) and Xk-related protein 8 (Xkr8) respectively. In particular, PS is mediated directly via one or more PS recognition receptors e.g. brain-specific angiogenesis inhibitor 1 (BAI1), cluster of differentiation 36 (CD36), and kidney injury molecule 1 (KIM1) or by soluble bridging molecules (indirectly) that bind PS on the apoptotic cells and a receptor on the phagocytes such as MFG-E8 [31]. It is noteworthy that PS is not completely absent from living cells, with an adequate threshold level of PS exposure being required to start engulfment [32]. Initiation of apoptosis alters "don't eat-me" signals expression, such as CD31[33], CD46 [34], and CD47 [35], which can further favor the apoptotic cells uptake.

Once ligand-receptor interaction occurs, cytoskeletal rearrangement takes place to initiate the internalization process via the ELMO-DOCK-Rac1 activation complex [44]. ELMO, a homologue CED-12 protein in *C. elegans*, is a well-known protein which modulates the activities of other proteins and does not have intrinsic catalytic activity. ELMO is an upstream regulator of Rac1 and regulated by BAI1 [40], Arhgef16 [44] and RhoG [45]. Dock1 as a guanine-nucleotide exchange factor (GEF) catalyses the exchange of guanine diphosphate (GDP) (inactive form) for guanine triphosphate (GTP) to activate Rac1. Myosin-II is the target of Rac1 to modulate actin assembly [44]. Instead of

pseudopodia protrusion like in macrophage cells, Parnaik et al [46] noticed via time-lapse video recording, that fibroblast cell displayed intermittent membrane ruffles over several hours in the cell-cell contact area, moving the apoptotic cells around on the membrane and abruptly engulfing them. In contrast, through electron microscope observation, Wagner et al [25] showed that human peritoneal mesothelial cells (HMCs) formed a pseudopod structure to engulf fragmented cells. Moreover, endothelial cells also show similar capacity to protrude cytoplasmic pseudopodia into the capillary lumen [47]. The mechanism of swallowing cellular debris is highly dependent on the distinct nature of the APs.

One understudied aspect of phagocytosis is the influence of *mechanics* on a cell's ability to engulf material. For example, the stiffness of the cell is likely to impact on the ability to wrap around its target, while shear stress imparted by interstitial flow could influence the receptor-ligand binding process. Indeed, it has recently been shown that phagocytosis of *Leishmania* parasites is hindered by the presence of flow [48]. While the precise mechanism has yet to be elucidated, it seems highly plausible that a combination of the forces imparted by flow along with the spatio-temporal gradients of chemicals and nutrients that flow supplies are likely to be key determinants of whether or not a cell will engulf material. Thus the specific flow environment experienced by cells, may well go some way to explaining the differential role of APs depending on location within the body.

Fascinatingly, while professional macrophages identify and engulf cells at an early stage of apoptosis, APs engulf only pre-aged apoptotic cells. The nature of this difference is unclear, nevertheless it indicates that time is a critical factor for apoptotic cells to acquire specific features, probably concentration of externalized PS and "eat-me" signals, for internalization by APs. Another possibility may be that more than one "find-me" signal is required for to apoptotic cell uptake, as reflected in two cases with professional phagocytes [49,50].

Following cellular debris compartmentalization, GTPase Rab5 [51] attaches to the nascent phagosome or efferosome to mediate the fusion of early endosome via Rab5 effectors. Rab5 is exchanged for Rab7 to bridge the fusion of late endosome and lysosome to form phagolysosome (ply), indicated by lysosomal-associated membrane protein 1 (LAMP1) expression [52], in both membrane-bound vesicles. The process of phagosomal acidification occurs first, then followed by ply –formation: similar processes are observed in professional phagocytes [53].

Unlike the mechanism mentioned above, a recent study [4] performed in our lab showed that myelin cell debris was opsonized by IgG due to the lack of a specific ligand (*naked myelin debris*) to bind the specific MVECs receptor. IgG was recruited to the myelin debris and engaged the Fcγ receptor, leading to cellular debris packaging and shipment via the autophagy pathway. Based on RNA sequencing, we observed the enhanced expression of several autophagy genes such as GABARAPL2, GABARAP, Atg12, LC3b, Atg5, and Atg3. This evidence suggests activation of autophagy. Furthermore, microtubule-associated proteins 1A/1B light chain 3b (LC3b), one of three splice variants of LC3, was detected and played a central role in elongation of the phagophore membrane, whereas GABARAP is required for autophagosome maturation. Additionally, autophagosome membrane expansion and fusion were also observed, indicated by conversion of cytostolic LC3-I to LC3-II. These genes are also involved in the autophagy mechanism in professional macrophages, warranting further investigation to confirm the similarity of autophagy in professional and amateur phagocytes.

The Implication of phagocytosis by APs

Apoptotic body released by dying cells carries bioactive molecules, such as RNA, lipid, and peptide molecules that can affect the surrounding cells either via paracrine or autocrine manner. In response to atherosclerosis, endothelial cell-derived apoptotic bodies carry microRNA-126 (miR-126) which then transferred to the recipient cells and convey paracrine signals to trigger CXCL12 production. As the consequence, CXCL12 promotes progenitor cells mobilization to the injured area, thus it creates plaque stability and acts as anti-apoptosis factor [54]. Another study shows that microparticles from endothelial cells can protect acceptor cells from apoptosis by inhibiting p38 activity [37]. Furthermore, circulated cellular debris in the blood promotes the attachment of platelets due to the von Willebrand factor (vWF) expression at the HUVEC surface and involvement of glycoprotein Ib and P-selectin. Debris is then internalized by ECs and produces reactive oxygen species (ROS) via the xanthine/xanthine oxidase system and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. In a patient study, it was shown that platelet aggregation correlates with the severity of vascular lesion in type I diabetic patients [55]. In addition, there are many various cytokines released by APs after cellular debris engulfment and degradation which are summarized in Table 2.

Generally, the uptake of apoptotic cells produces anti-inflammatory responses (TGF-β and IL-10) and decreased secretion of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-8) whereas phagocytosis of necrotic cells commonly leads to pro-inflammatory signaling due to leakage of cellular material to the cell environment (William et al, 2000, Sexton et al, 2001). However, APs elicit two different cytokines after engulfing apoptotic bodies: 1) pro-inflammatory cytokines which activate endothelial cells and promote leucocyte sequestration, thus modulating an inflammatory response and 2) anti-inflammatory cytokines. Phagocytic clearance of apoptotic cells by kidney epithelial cells subsequently downregulated NF-κB activity resulting in an anti-inflammatory phenotype in proximal tubular cells (PTCs), including reduced toll-like receptor 4 (TLR4) expression, pro-inflammatory cytokine production, and a decreased ability to activate macrophages [2]. Moreover, apoptotic Jurkat cell engulfment by LR73 cells and peritoneal macrophages share similar transcriptional profiles such as decreased expression of pro-inflammatory genes, increased expression of actin rearrangement/cell motility genes, and increased expression of anti-inflammatory genes [56]. In contrast, a recent study showed that engulfment of myelin debris by microvascular endothelial cells stimulates an inflammatory response, promotes inflammation by inducing leukocyte infiltration and triggers endothelial cells angiogenesis [4]. Similarly, Krisch et al [36] found that engulfment of apoptotic ECs by a human microvascular endothelial cell line (HMVEC-1) resulted in increased expression of pro-inflammatory chemokines and enhanced binding of leukocytes to HMVEC-1 cells. The discrepancy between two different results (anti-inflammatory vs pro-inflammatory response) is probably due to the difference of cell sources, activation mechanisms, and preparation methods. The composition of apoptotic bodies or cellular debris is highly related to their cellular origin and to the type of stimulus involved in their formation. Therefore, the cellular events caused by their engulfment by APs may also be different. Hadda-Berda et al [39] showed that apoptotic HUVECs had two different forms, microparticles (described as < 1 μm, $AnnexinV^{+}/DAPI^{-}/histone^{-}$) and apoptotic bodies, AptB, (1-3 μm, $AnnexinV^{+}/DAPI^{+}/histone^{+}$). AptB contained IL-1α and were able to induce chemokine IL-8 and MCP-1, whereas microparticles were lacking of IL-1 α and thus incapable of inducing pro-inflammatory chemokines.

Interestingly, cellular debris engulfment could also induce fibroblast-like cells via a phenotype resembling endothelial-to-mesenchymal transition (endoMT). Thus, brain microvascular endothelial cells (BMECs) treatment with strong inducer endoMT TGF-β1 and myelin debris lead to BMEC elongation and indicate the occurrence of an endothelial derived fibrotic component (Zhou et al, 2019). Brock et al [57] reported that epithelial stem cells that

223 engulf the epithelial stem cell-derived apoptotic bodies (ESABs) through activation of Wnt signalling regulate
224 proliferation of healthy stem cells and maintains epithelial tissue homeostasis. In addition, Liu et al [2] in their study
225 used Fas deficient MRL/lpr and Caspase $3^{-/-}$ mice and also found that mesenchymal stem cells (MSCs) were able to
226 engulf apoptotic bodies which were important in maintaining MSCs properties and bone homeostasis, thereby
227 highlighting the potential of apoptotic bodies for treating osteoporosis.
228
229 In addition to the lysosome to degrade the cellular deris, mitochondria play a pivotal role indirectly enhancing in
230 cellular debris clearance. A recent discovery [58] in macrophage cells reveals that mitochondria assists apoptotic
231 cell clearance by dynamin related protein (Drp-1) mediated mitochondrial fission which allows efficient apoptotic
232 cell degradation in the phagolysosome as well as endoplasmic reticulum Ca^{2+} release into the cytoplasm to mediate
233 vesicular trafficking for phagocytosis of a secondary apoptotic cell. Not only does the lysosome break down foreign
234 material, but it also makes contact with mitochondria to regulate mitochondrial fission via RAB7 GTP hydrolysis. It
235 is important to note that during this process, mitochondria are not engulfed by the lysosome [59]. This finding leads
236 to some fundamental questions: are there any cellular organelles involved and what is their role in cellular debris
237 clearance? Do mitochondria also contribute to continued clearance of apoptotic cells in AP? If yes, then is there a
238 link between mitochondria fission and EndoMT? It is known that fragmented mitochondria is associated with a
239 cellular reprogramming event which is a hallmark of stem cell [60,61]. In terms of metabolic activity, engulfment of
240 apoptotic cells by AP enhances glycolysis metabolism which is the primary means of producing energy for stem
241 cells. Compared to professional phagocytes (PPs), efferocytic macrophages are reported to be oxidative
242 phosphorylated-dependent [62] while AP show downregulated oxidative phosphorylation and fatty acid oxidation
243 expression [63,56].
244
245 **Conclusion and Future Perspective**
246 Cellular debris has a double-edged sword feature, it is required for homeostasis but also contributes to disease
247 progression. Recent studies have revealed the role and existence of APs as a main player or assistant of professional
248 macrophages during cellular debris removal. Cellular debris presence in APs can activate two different signaling
249 pathways, efferocytosis/phagocytosis and opsonization: however it remains unclear how APs determine these
250 mechanisms. It has been suggested that “naked” debris is opsonized by IgG (opsonization), whereas apoptotic

bodies rely on specific ligand-receptor engagement (phagocytosis/efferocytosis). Furthermore, the content of apoptotic is largely understudied and should be explored as it determines the response of APs. The indirect role of cellular organelles (endoplasmic reticulum and golgi apparatus) is essential during vesicle formation, and so it is appealing to dissect the hidden potential of cellular organelles, like mitochondria [58], in removing cellular debris. There are many open questions regarding cellular debris engulfment and clearance e.g. the mechanism to engulf cellular debris in different APs, how recycling nutrients contributes to the cytokine release and cellular reprogramming, and understanding crosstalk with innate and adaptive immune system. Moreover, the role of mechanical cues such as stiffness and shear stress from fluid flow deserve further attention, as it seems certain that they too will play an important role.

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Competing interests

The authors declare no competing interests.

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Figure Captions

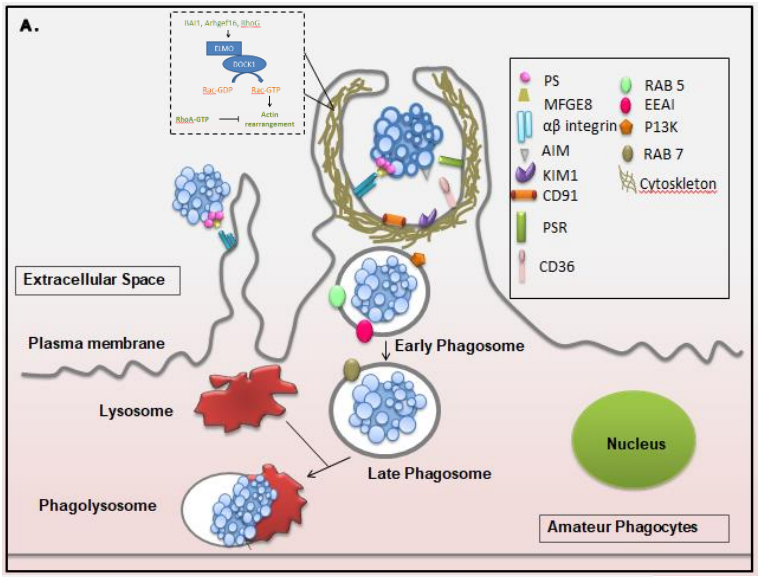
Figure 1. Cell debris engulfment and clearance in Aps due to intercellular, transmembrane, and intracellular signaling. Proposed model of cellular debris engulfment by membrane ruffle, initiated by probing structure formation followed by circular ruffle surrounding the cellular debris or pseudopodia structure (A). There are two mechanisms of cellular debris internalization (A) phagocytosis/efferocytosis pathway and (B) autophagy pathway. Cell engulfment is initiated by introducing ligands to bind to the receptors (A) or IgG opsonization (B). Then, cell debris is compartmentalized within a specific cargo that originates from the plasma membrane in the form of the phagosome (phagosome pathway) (A) or endoplasmic reticulum in the form of phagophore (autophagy pathway) (B). Both processes involve different proteins to maturate the cargo and require lysosome to degrade its compartment.

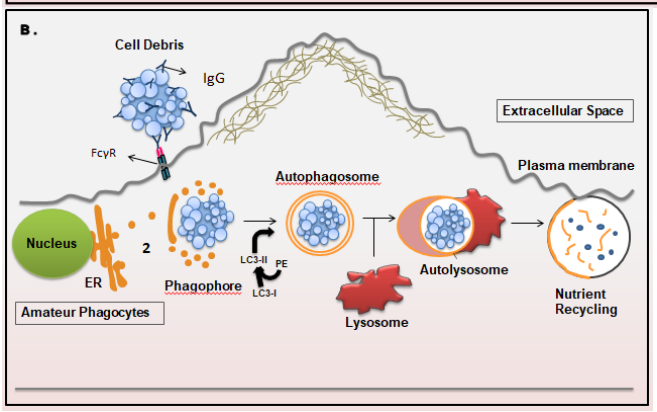
Table Captions

Table 1. Types of ligand-receptor pairs within different non-professional phagocytes

Table 2. Cytokine release after cell engulfment by APs

Figure 1


A.
PS
MFGE8
αβ integrin
AIM
KIM1
CD91
PSR
CD36
RAB 5
EEAI
P13K
RAB 7
Cytoskleton
Extracellular Space
Plasma membrane
Early Phagosome
Lysosome
Late Phagosome
Nucleus
Phagolysosome
Amateur Phagocytes


B.
Cell Debris
IgG
FcγR
Extracellular Space
Plasma membrane
Autophagosome
Nucleus
ER
2
Phagophore
LC3-II
PE
LC3-I
Autolysosome
Lysosome
Nutrient Recycling
Amateur Phagocytes

487 **Table 1**.

Cell type	Source of debris	Ligand	Receptor	Ref.
Human umbilical vein endothelial cells (HUVECs) and microvascular endothelial cells	Platelets	PS	$\alpha_v\beta_3$	[36]
Human coronary artery endothelial cells (HCAEC)	HCAEC	PS	PS receptor (PSR)	[37]
Human brain endothelial cells	Platelets	PS	-	[38]
HUVECs	Acute promyelocytic leukemia	PS, MFG-E8	αβ	[39]
Brain MVECs	Myelin debris	Immunoglobulin G (IgG) (opsonisation)	FcγR	[4]
Colonic epithelial cells	*In vivo* study by inducing colitis disease	PS	BAI1	[40]
Bronchial epithelial cells	*In vivo* study using endotoxin house dust mite (HDM) extract and chicken Ovalbumin (allergens)	PS	-	[26]
Alveolar epithelial cells	Eosinophils	PS	CD36, $\alpha_v\beta_3$, $\alpha_v\beta_5$	[27]
Mammary alveolar epithelial cells	Alveolar epithelial cells	PS	CD91, CD36, $\alpha_v\beta_3$	[41]
Kidney epithelial cells	*In vivo* study with acute kidney injury model	PS	KIM1	[42]
Kidney epithelial cells	*In vivo* study with acute kidney injury model	AIM	KIM1	[28]

Smooth muscle cells	Erythrocytes	PS	PSR	[43]
Myofibroblast	cardiomyocytes from myocardial infarction	MFG-E8	$\alpha_v\beta_3$	[5]

- : not mentioned

Table 2.

Source of debris	Cell engulfment	Type of programmed cell death	Cytokine	Ref
Murine erythrocytes	Mouse pulmonary ECs	Apoptotic	↑ELAM1, ↑ICAM1, ↑IL6	[64]
Human microvascular endothelial cells (HMVECs)	HMVEC1, primary HUVECs	Apoptotic and necrotic	↑IL8, ↑MCP1	[36]
Aged murine erythrocytes	Murine lung endothelial cells (MLECs)	-	↑IL6, ↑E-Selectin, and ↑ICAM	[51]
Plasma microparticles from human	Human aortic endothelial cells (HAECs) (*additional treatment with VEGFi*)	-	↑ET1, ↓NO, ↑TNF-α, ↑IL-6, ↑MCP-1, ↑iNOS, ↑COX-2, and ↑VCAM-1	[65]
T-lymphocytes or from diabetic patients	Human umbilical artery smooth muscle cells (HUASMCs)	Apoptotic	↑NO, ↑prostacyclin, ↑iNOS, ↑COX-2, ↑NF-kB	[66]

T-cells	HUVECs	Apoptotic	↓NO, ↓Prostacyclin, ↑Caveolin-1	[67]
HC-11, Jurkat T cells, and PLB 985 cells	Epithelial cell lines (HC-11, EpH4, and PMEC)	Apoptotic	↑TGFβ	[41]
Epithelial cells	MLE12, BEAS-2B, CCSP-Cre/Rac1$^{fl/fl}$ mice	Apoptotic	↑TGFβ, ↑PGE_2 ↑IL10, IL33↓ (anti-inflammatory)	[26]
Myelin debris	BMECs, bEnd.3	-	↑VCAM, ↑Fibronectin, ↑Collagen	[4]
Colonic epithelial cells	Apoptotic epithelial cells *(externally induced by dextran sulfate sodium)*	Apoptotic	↑IL-1α, IL-33, and TNF	[24]
Kidney epithelial cells	Luminal cellular debris	Apoptotic	NF-kB ↓ TLR4↓	[68]

- : Not mentioned

Abbreviation:

- ELAM 1 : Endothelial-Leukocyte Adhesion Molecule 1
- ICAM1 : Intercellular Adhesion Molecule 1
- MCP1 : Monocyte Chemoattractant Protein 1
- ET1 : Endothelin 1
- NO : Nitric oxide
- iNOS : Inducible Nitric Oxide Synthase
- COX-2 : Cyclooxygenase-2
- VCAM : Vascular Cell Adhesion Molecule
- TGF-β : Transforming Growth Factor- β

- PGE_2 : Prostaglandin E_2
- TNF : Tumor Necrosis Factor
- NF-kB : Nuclear Factor-Kappa Beta
- VEGFi : Vascular Endothelial Growth Factor Inhibitor