Review

The clearance of dying cells: table for two

DR Green1, TH Oguin2 and J Martinez*2

Phagocytic cells of the immune system must constantly survey for, recognize, and efficiently clear the billions of cellular corpses that arise as a result of development, stress, infection, or normal homeostasis. This process, termed efferocytosis, is critical for the prevention of autoimmune and inflammatory disorders, and persistence of dead cells in tissue is characteristic of many human autoimmune diseases, notably systemic lupus erythematosus. The most notable characteristic of the efferocytosis of apoptotic cells is its ‘immunologically silent’ response. Although the mechanisms by which phagocytes facilitate engulfment of dead cells has been a well-studied area, the pathways that coordinate to process the ingested corpse and direct the subsequent immune response is an area of growing interest. The recently described pathway of LC3 (microtubule-associated protein 1A/1B-light chain 3)-associated phagocytosis (LAP) has shed some light on this issue. LAP is triggered when an extracellular particle, such as a dead cell, engages an extracellular receptor during phagocytosis, induces the translocation of autophagy machinery, and ultimately LC3 to the cargo-containing phagosome, termed the LAPosome. In this review, we will examine efferocytosis and the impact of LAP on efferocytosis, allowing us to reimagine the impact of the autophagy machinery on innate host defense mechanisms.

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Facts

- Efferocytosis is a carefully orchestrated process by which phagocytes are recruited to sites of cell death, recognize and engulf dying cells, and clear them in an ‘immunologically silent’ manner.
- Dying cells have an active role in their own clearance; via the production of ‘find-me’ signals to attract phagocytes and exposure of ‘eat-me’ signals that engage phagocytic receptors to facilitate engulfment.
- Defects in the efferocytosis machinery are associated with inflammation and autoimmune disorders, such as systemic lupus erythematosus (SLE).
- Microtubule-associated protein 1A/1B-light chain 3 (LC3)-associated phagocytosis (LAP) is required for the effective clearance of dying cells.
- Do defects in LAP contribute to inflammatory or autoimmune pathogenesis?
- What role does LAP have in oncogenesis? What role does LAP have in tumor-associated macrophages?

Open Questions

- Given the variety of ‘find-me’ and ‘eat-me’ signals, as well as their cognate receptors, how do these signals coordinate for effective efferocytosis?
- How does LAP promote the anti-inflammatory response to dying cells, and what role does macrophage metabolism have?
- An Introduction: Can I Interest You in Any Appetizers?

Even from our earliest developmental stages, the process of generating and maintaining a multicellular, functional organism is characterized by the creation and unceremonious destruction of billions of cells.1 Programmed cell death, such as apoptosis, necroptosis, or pyroptosis, are active mechanisms designed to sculpt, control, and aid the body in its development and survival. Much of our knowledge on the role of apoptosis in development comes from the study of Caenorhabditis elegans, wherein the first wave of cell death occurs ~4 h after fertilization, and of the 1090 cells that are generated, 131 of them are destined for death.2 In mammalian embryos, apoptosis is seen during caviation and has a dynamic role in shaping the embryo.3 It is now well understood that proper apoptosis is fundamental for the proper development of the organism, as deficiencies in mediators of apoptosis result in embryonic lethality or animals with severe malformations.4 Conversely, other forms of cell death, such as necroptosis and pyroptosis, are not required during

1Department of Immunology, St. Jude Children’s Research Hospital, Memphis, TN, USA and 2Immunity, Inflammation, and Disease Laboratory, NIH/National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
*Corresponding author: J Martinez, Immunity, Inflammation, and Disease Laboratory, NIH/National Institute of Environmental Health Sciences, 111 T. W. Alexander Dr., MD D2-01, Research Triangle Park, NC 27709, USA. Tel: 919 541 4420; Fax: 301 480 2317; E-mail: jennifer.martinez3@nih.gov

Abbreviations: ATG, autophagy-related gene; DAMP, danger associated molecular pattern; IC, immune complex; IRF, interferon regulatory factor; LAP, LC3-associated phagocytosis; LC3, microtubule-associated protein 1A/1B-light chain 3; LXR, liver X receptor; PI(3)P, phosphatidylinositol 3-phosphate; POS, photoreceptor outer segment; PPAR, peroxisome proliferator-activated receptor; PtdSer or PS, phosphatidylserine; ROS, reactive oxygen species; RPE, retinal pigment epithelium; SLE, systemic lupus erythematosus; TAM, Tyro-3, Axl, and Mer; TLR, Toll-like receptor

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development, as evidenced by the normal development of mice deficient for receptor interacting protein kinase3 or caspase-1/11, respectively.5,6 Once formed, the organism’s relationship with cell death is far from over. Cellular turnover is a constant, genetically programmed process in the adult animal, and removal of these unwanted and unneeded cellular corpses is vital to prevent unwanted inflammation and immune activation.7 Although damage can certainly cause unwanted cellular death, most cellular death is an active process, and perturbations in the cell death programs can promote cell accumulation, autoimmunity, oncogenesis, attrition, and/or degeneration.

Within tissues, professional, non-professional, and specialized phagocytes are tasked with the clearance of dying cells. The best-characterized population of professional phagocytes, macrophages, is composed of tissue-specific, differentiated subsets of resident macrophages that clear dying cells and debris.8 For example, Kupffer cells in the liver clear aged red blood cells;9 alveolar macrophages of the lung clear apoptotic airway epithelial cells,9 and microglia in the central nervous system clear dying neurons.10 Other types of resident cells, such as epithelial cells and fibroblasts, have been termed non-professional phagocytes; though this designation may be a misnomer as these cells have a major role when professional phagocytes are rare, such as in the mammary gland or intestinal epithelium. In addition, airway epithelial cells are critical for the clearance of apoptotic airway epithelial cells, and epithelial cell-specific deletion of Rac1 results in increased allergen-induced airway inflammation.11 Still other types of tissue-specific, multifunctional cells exist as specialized phagocytes. In the testes, Sertoli cells are responsible for clearing apoptotic germ cells that arise during spermatogenesis.12 In the eye, retinal pigment epithelial (RPE) cells are critical for the homeostatic, daily removal of the photoreceptor outer segments (POSs), and the generation of 11-cis-retinal for the visual cycle. Defects in RPE cell-mediated removal of outer segments (or processing of outer segments via LAP, discuss below) can lead to a predisposition to conditions, such as age-related macular degeneration or retinitis pigmentosa.13

Like the death process itself, the innate immune system has tolerance systems in place to manage these morbidity events. Although the generation and subsequent destruction of these cells is necessary for normal cellular homeostasis, wound healing, and immune responses in the adult organism, the ruin left in its wake would be catastrophically not for the efficient work of the phagocytic system.14 Despite the constant, homeostatic turnover of cells, as well as cell death induced by stress, damage, or infection, it is rare to observe apoptotic cells under normal physiological conditions. Considering the average one million adult human cells that undergo apoptosis every second, one can appreciate the magnitude of the job facing phagocytes15 Moreover, as this is a reoccurring and normal event in the lifespan of an organism, this process of dead cell clearance must occur in a quiescent manner, so as to not inappropriately alert the immune system.16

We now appreciate the critical role that efferocytosis has on modulating immunity, as well as the impact that different types of cell death have on the immune response. In this review, we discuss the process of efferocytosis, chemoattraction of phagocytes, recognition of dying cells, engulfment of cellular corpses, and the processing of engulfed cellular cargo, specifically the role of LAP in clearance of dying cells and control of inflammation. Finally, we explore the effect of defective efferocytosis on pathology and disease states.

The Mechanisms of Efferocytosis: Would You Like to Hear the Specials?

As the focus of this review is the aftermath of cell death, we have summarized the four most well-defined modes of cell death (apoptosis, necrosis, necroptosis, and pyroptosis) in Table 1, as the roles and mechanisms of cell death have been studied and reviewed extensively.1

Efferocytosis is not merely a passive event, but a carefully orchestrated process designed to efficiently eliminate cellular corpses and limit exposure to their potentially damaging components, with the goal being immunological tolerance.17 Efferocytosis 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. We now recognize that defects in any of these four steps can contribute to unwanted inflammation and autoimmune disorders, such as systemic lupus erythematosus18 (Table 2).

As phagocytes are often not proximal to sites of cell death or even reside in the tissues they must survey, dying cells must ‘advertise’ their presence to phagocytes.19 Recruitment of phagocytes to sites of cell death in C. elegans occurs before the completion of apoptosis, indicating that one of the first acts of a dying cell is to prepare for its own elimination.20,21 During this process, apoptotic cells release ‘find-me’ signals, distinct molecules that establish a chemotactic gradient to attract phagocytic cells.22 Nucleotides, such as ATP, are released in a caspase-dependent manner via activation of pannexin-1 channels and are perhaps the most well-defined ‘find-me’ signals.23 These nucleotides are detected by phagocytes via purinergic receptors, like P2Y2, and disruption of the nucleotide/P2Y2 interaction results in an accumulation of dying cells in vivo.19 Apoptotic cells also release the membrane-associated molecule fractalkine (or CX3CL1), which is sensed by CX3CR1 and mediates the migration of macrophages to the dying cells. Mice deficient for CX3CR1, however, do not display a defect in apoptotic cell clearance, suggesting that multiple factors are required to recruit effectively phagocytes.24 Molecules of lipid origin can also act as ‘find-me’ signals. Lysoosphatidylcholine is generated and released via caspase-3-dependent activation of phospholipase A, and is sensed by the G-protein-coupled receptor G2A.25 Sphingosine-1-phosphate (S1P), also a lipid ‘find-me’ signal, is released by dying cells and sensed by multiple G-protein-coupled receptors S1P-R1-5. These lipid signals have been demonstrated to mediate phagocyte chemotaxis26 (Figure 1a).

There are significant caveats to the ability of ‘find-me’ signals to efficiently act as chemoattractants in physiologically scenarios. The idea of an apoptotic cell’s purposeful release of
Summary of the four major modes of cell death: apoptosis, necrosis, necroptosis, and pyroptosis

| Description | Characteristics | References |
|-------------|-----------------|------------|
| **Apoptosis** | Active cellular death, largely controlled by a family of cysteine proteases called caspases | Membrane 'blebbing', often with separation of apoptotic bodies and DNA fragmentation | 1,99–103 |
| Intrinsic or mitochondrial pathway | Activated by stress-inducing stimuli (i.e., DNA damage, accumulation of unfolded proteins, hypoxia) and developmental signals | DNA fragmentation | 1,99–103 |
| | Signals converge on the mitochondria, where pro-apoptotic and anti-apoptotic members of the BCL2 family mediate the release of cytochrome c, formation of the apoptosome with caspase-9 and APAF-1, which leads to the activation of the downstream executioner caspases, such as caspase-3 and caspase-7 | Chromatin condensation | 1,104–106 |
| Extrinsic pathway | Triggered by signals that engage extracellular death receptors (DR) | Cellular swelling (oncosis) and Organelle swelling | 1,104–106 |
| | Tumor necrosis factor (TNF) and TNF receptor-1 (TNFR1) | Cellular rupture | 5,107–112 |
| | CD95-ligand (CD95-L or Fas-L) and CD95 (or Fas) | Releases inflammatory cellular contents (DAMPs) or alarmins that can activate neighboring immune cells via Toll-like receptor (TLR) signaling and other mechanisms | 5,107–112 |
| | TNF-related apoptosis-inducing ligand (TRAIL) and TRAIL-R1/2 (DR4/5) | Annexin V positive due to membrane rupture | 5,107–112 |
| | Recruitment of pro-caspase-8 to the death-inducing signaling complex (DISC) at the DR (with the adapter proteins FADD or TRADD), resulting in dimerization and activation of caspase-8, leading to caspase-3 and caspase-7 activity | Propidium iodide or 7-AAD positive at early stages | 5,107–112 |
| | Caspase-8 activity can also feed into the intrinsic pathway by cleaving and activating BCL2 family proteins | | |

| **Necrosis** | Characterized as an passive type of cell death that occurs independently of caspase activation | Cellular swelling (oncosis) and Organelle swelling | 1,104–106 |
| | Triggered in response to catastrophic damage or pathology, including infarction, mechanical trauma, ischemia, frostbite, and animal venom | Cellular rupture | 5,107–112 |
| | Apoptotic cells that are not efficiently cleared by phagocytes can undergo secondary necrosis independently of any apoptotic machinery | Releases inflammatory cellular contents (DAMPs) or alarmins that can activate neighboring immune cells via Toll-like receptor (TLR) signaling and other mechanisms | 5,107–112 |
| | | Annexin V positive due to membrane rupture | 5,107–112 |
| | | Propidium iodide or 7-AAD positive | 5,107–112 |
| **Necroptosis** | Genetically programed cell death with the morphological features of necrosis | Loss of plasma membrane integrity and Swollen cellular organelles | 1,4,6,51,113,114 |
| | Triggered by diverse forms of neurodegeneration, ischemia, or infection. Engagement of the extrinsic pathway (i.e., TNF–TNFR pathway) in the absence of caspase-8 can result in a necrotic cell death that requires the kinase activity of receptor interacting protein kinases 1 (RIPK1) and RIPK3 | Releases inflammatory cellular contents (DAMPs) or alarmins that can activate neighboring immune cells via Toll-like receptor (TLR) signaling and other mechanisms | 1,4,6,51,113,114 |
| | RIPK3 phosphorylates and activates the pseudokinase, mixed lineage-kinase like (MLKL) | Active PtdSer exposure (Annexin V positive) | 1,4,6,51,113,114 |
| | Induces a conformational change that allows for its oligomerization and interaction with the plasma membrane through binding to phosphatidylinositol lipids to directly disrupt membrane integrity | Propidium iodide or 7-AAD positive | 1,4,6,51,113,114 |
| | RIPIK1 is required for a variety of innate immune signaling pathways, such as TLRs, interferons, and the RIG-I-MAVS pathway | | |

| **Pyroptosis** | Non-apoptotic, genetically programmed cellular death mediated by excessive inflammatory caspase activity (human caspase-1, -4, and -5; rodent caspase-1 and -11) | Cellular rupture and DNA fragmentation | 1,4,6,51,113,114 |
| | Required for cell death in a variety of experimental settings, including in the immune system, the cardiovascular system, and the central nervous system | Releases inflammatory cellular contents (DAMPs) or alarmins that can activate neighboring immune cells via Toll-like receptor (TLR) signaling and other mechanisms | 1,4,6,51,113,114 |
| | Caspase-1 is activated by dimerization at complexes termed inflammasomes that form in the cytosol and detect a diverse repertoire of pathogenic molecules, including bacterial toxins and viral RNA | Often Annexin V positive due to membrane rupture | 1,4,6,51,113,114 |
| | Activated caspase-1 in turn cleaves pro-IL-1β and pro-IL-18, which facilitates the secretion of these pro-inflammatory cytokines | Propidium iodide or 7-AAD positive | 1,4,6,51,113,114 |
| | Characterized by caspase-1-dependent formation of plasma membrane pores, which leads to pathological ion fluxes that ultimately result in cellular lysis and release of inflammatory intracellular contents | | |
| | Caspase-1 can also activate caspase-7 | | |

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Indeed, <2% of intracellular ATP is released during apoptosis.\textsuperscript{15} In addition, these released nucleotides must also survive degradation by extracellular nucleotidases, indicating that they most likely act in a short-range capacity.\textsuperscript{19} Similarly, 'find-me' signals of lipid origin are present ubiquitously in the circulation at a concentration higher than that released by apoptosis.\textsuperscript{34} Caspase 3-mediated cleavage of the scramblase proteins, which can be recognized by a wide variety of cells, specifically recruit phagocytes, the majority of which are macrophages, are unknown. The counteractive effect of 'keep out' signals has been proposed to aid in the coordinated recruitment of phagocytes. For example, lactoferrin, a glycoprotein released by apoptotic cells, has been shown to actively exclude neutrophils and eosinophils from sites of cell death.\textsuperscript{28,29} Further complicating the matter is the dual role that they most likely act in a short-range capacity.\textsuperscript{19} Similarly, extracellularly exposed lipid, phosphatidylserine (PtdSer), is rapidly externalized in a caspase-dependent manner during apoptosis.\textsuperscript{34} The clearance of dying cells is inactivated by caspase-3 cleavage.\textsuperscript{36} Extracellularly exposed PtdSer is recognized by multiple, bona fide receptors, such as integrin \( \alpha v \beta 5 \), or Tryo3-Axl-Mer (TAM) receptors, which are unknown. The counteractive effect of 'keep out' signals has been proposed to aid in the coordinated recruitment of phagocytes. For example, lactoferrin, a glycoprotein released by apoptotic cells, has been shown to actively exclude neutrophils and eosinophils from sites of cell death.\textsuperscript{28,29} Further complicating the matter is the dual role that they most likely act in a short-range capacity.\textsuperscript{19} Similarly, extracellularly exposed lipid, phosphatidylserine (PtdSer), is rapidly externalized in a caspase-dependent manner during apoptosis.\textsuperscript{34} The clearance of dying cells is inactivated by caspase-3 cleavage.\textsuperscript{36} Extracellularly exposed PtdSer is recognized by multiple, bona fide membrane receptors, such as T-cell immunoglobulin mucin receptor 4 (TIM4), brain-specific angiogenesis inhibitor 1 (BAI1), and stabilin-2.\textsuperscript{37,39} and bridging molecules, such as milk fat globule-EGF factor 8 (MFG-E8) and Gas6, that recognize PtdSer and then engage phagocytic cell surface receptors such as integrin \( \alpha v \beta 3 \), \( \alpha v \beta 5 \), or Tryo3-Axl-Mer (TAM) receptors\textsuperscript{40–42} for engulfment.

### Table 2 Components of the efferocytosis machinery and their association with inflammatory and autoimmune diseases

| Molecules associated with increased incidence or risk of disease | Role in efferocytosis | Disease(s) | References |
|---------------------------------------------------------------|----------------------|------------|------------|
| Nucleotides (ATP/UTP) | ‘Find-me’ | MS/EAE | 115 |
| Pannexin-1 | ‘Find-me’ | MS/EAE, seizure disorders | 116,117 |
| S1P | ‘Find-me’ | MS/EAE | 118 |
| LPC | ‘Find-me’ | Atherosclerosis, SLE/systemic autoimmunity | 119 |
| S1PR1-5 | ‘Find-me’ | MS/EAE | 118 |
| G2A | ‘Find-me’ | SLE/systemic autoimmunity, atherosclerosis | 120,121 |
| CX3CR1 | ‘Find-me’ | Autoimmune uveitis, MS/EAE | 122,123 |
| ICAM3 | ‘Eat-me’ | RA, SLE/systemic autoimmunity, GBS, MS/EAE | 124 |
| C1q | ‘Eat-me’ | RA, Sjögren’s syndrome, Celiac disease, SLE/systemic autoimmunity | 125 |
| TIM1 | ‘Eat-me’ | SLE/systemic autoimmunity, airway inflammation | 127,128 |
| TIM3 | ‘Eat-me’ | Airway inflammation, MS/EAE | 128 |
| TIM4 | ‘Eat-me’ | SLE/systemic autoimmunity | 37 |
| BA11 | ‘Eat-me’ | Glioblastoma, neurological disorders | 129 |
| Integrins (avβ3) | ‘Eat-me’ | Scleroderma, ulcerative colitis | 52,130 |
| MerTK | ‘Eat-me’ | SLE/systemic autoimmunity, retinitis pigmentosa, atherosclerosis | 53,131,132 |
| MFG-E8 | ‘Eat-me’ | SLE/systemic autoimmunity, atherosclerosis | 31,133 |
| ProteinS | ‘Eat-me’ | Thrombosis, SLE/systemic autoimmunity | 134,135 |
| CD300f | ‘Eat-me’ | SLE/glomerulonephritis | 136 |
| ELMO1 | Engagement | Testicular pathology, impaired neurogenesis | 137 |
| DOCK180 | Engagement | Cardiovascular abnormalities, myogenesis abnormalities | 138,139 |
| GRK6 | Engagement | SLE/systemic autoimmunity | 140 |
| RAC1 | Engagement | RA, airway inflammation | 11,141 |
| DNase II | Processing | Polyarthritides | 72 |
| LXRα/β | Processing | MS/EAE, SLE/systemic autoimmunity, autoimmune uveitis, type I diabetes, atherosclerosis | 142–148 |
| PPARα/γ | Processing | MS/EAE, SLE/glomerulonephritis, atherosclerosis, osteoarthritis | 95,96,149–151 |
| ABCA1 | Processing | SLE/glomerulonephritis | 152 |

| Molecules associated with decreased incidence or risk of disease | Role in efferocytosis | Disease(s) | References |
|---------------------------------------------------------------|----------------------|------------|------------|
| Fractalkine (CX3CL1) | ‘Find-me’ | Sjögren’s syndrome, airway inflammation, RA | 153–155 |
| Purinergic receptors (P2Y2) | ‘Find-me’ | Sjögren’s syndrome, autoimmune uveitis | 156,157 |
| Integrins (avβ3) | ‘Eat-me’ | MS/EAE | 158 |
| CD91 (LRP) | ‘Eat-me’ | RA, SLE/systemic autoimmunity | 159 |
| RAGE | ‘Eat-me’ | MS/EAE, DTH | 160,161 |
| GAS6 | ‘Eat-me’ | Thrombosis, nephrotoxic nephritis, SLE/systemic autoimmunity | 162–164 |

**Abbreviations:** BA1, brain-specific angiogenesis inhibitor 1; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalitis; C1q, complement 1q; CRT, calreticulin; GBS, Guillain-Barré syndrome; LPC, lysoosphatidylcholine; LXR, liver X receptor; MFG-E8, milk fat globule-EGF factor 8; MS, multiple sclerosis; PPARα/γ, peroxisome proliferator-activated receptor α/γ; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; S1P, sphingosine-1-phosphate; TIM, T-cell immunoglobulin mucin receptor.
Other ‘eat-me’ signals have also been identified, which are likely to have a ‘tethering’ function, facilitating the above events. ICAM3 can bind to membrane-associated CD14 and externalized calreticulin bound to complement C1q can engage CD91 (or LRP1). Oxidized LDL-like moieties and glycosylated surface proteins can serve as ‘eat-me’ signals, binding to scavenger receptors and lectins respectively (Figure 1b).

Similar to the ‘find-me’/‘keep-out’ signal paradigm, there is evidence of a negative signal to discourage phagocytosis. Although PtdSer is considered a hallmark of cell death, forced PtdSer exposure or physiologically normal exposure on activated, living cells does not mediate recognition or engulfment. Thus, the simultaneous presence of ‘don’t-eat-me’ signals, such as CD31, CD47, and CD61, on viable cells, may negatively regulate phagocytosis, indicating to the phagocyte that despite the extracellular PtdSer, this cell is not intended for clearance. Therefore, a coordinated effort between the dying cell and the phagocyte must exist to facilitate efferocytosis.

Although the majority of work on ‘find-me’ and ‘eat-me’ signals stems from apoptotic cells, these signals also function during other types of cell death, such as necroptosis and pyroptosis. As previously mentioned, ‘find-me’ signals, such as ATP, are released (and in greater quantities) during necrosis, necroptosis, and pyroptosis. Similarly, necrotic and pyroptotic cells also stain positive for Annexin V, although in these cases, externalized PtdSer can be attributed to rupture of the plasma membrane rather than an active exposure process. These dead cells can still be recognized and engaged by PtdSer receptors, however, owing to the lytic nature of their demise, DAMPs have already been released into the milieu and can activate inflammatory programs. Therefore, although apoptotic cells actively coordinate their own clearance, necrotic and pyroptotic cells passively utilize these systems as well.

The tissue specificity of PtdSer receptors may help to explain why multiple receptors are required for efficient efferocytosis. Stabilin-2 is highly expressed in endothelial cells within atherosclerotic plaques, although defects in BAI1, highly expressed in glial and neuronal cells, are associated with neurodegenerative disorders. Despite a common ligand and a common goal of engulfment, the mechanisms by which PtdSer receptors mediate phagocytosis are often varied. Once engaged by PtdSer-bound integrins, bridging molecules, such as αβ3 or TAM, associates with the adapter proteins ELMO1 and DOCK180 (via CrkII) at the site of phagocytosis. The PtdSer receptor BAI1 also requires the activity of the DOCK180/ELMO1 complex for engulfment, but BAI1 is able to recruit the complex independently. Stabilin-2 and CD91/LRP, however, require the activity of the adapter protein, GULP, to facilitate phagocytosis. One of the most well-known PtdSer receptors, TIM4, contains a very short cytoplasmic region and currently its signaling components are unknown.

Dying cell engulfment involves active membrane ruffling by a process similar to macroinocytosis. Engagement of PtdSer receptors results in cytoskeletal reorganization to facilitate phagocytosis, which is mediated by the Rho family of GTPases, including RhoA, ROCK, Rac, Rab5, and Cdc42. These GTPases cycle between the resting, inactive GDP-bound state and the active GTP-bound state, mediated by specific guanine-nucleotide-exchange factors (GEFs), such as the bipartite GEF formed by DOCK180 and ELMO1. Ultimately, signaling during efferocytosis converges on the activation of evolutionarily conserved Rac1, acting at the phagocytic cup to promote actin polymerization and cytoskeletal rearrangement via the Scar/WAVE complex. Similarly, CDC42 has been linked to the engulfment of apoptotic cells, although its precise role is unclear. Once encased within the phagocyte, however, the dying cell is now capable of exerting its effect on critical downstream immunological and metabolic pathways.
in an immunologically tolerant manner, it must also contend with the excess lipid, cholesterol, and protein that an entire engulfed cell brings. Acidic proteases and nucleases in mature phagolysosomal compartments degrade dying cells into their basic cellular components, including fats, sterols, peptides, and nucleotides. For example, DNAse II, a lysosomal enzyme, is required for the degradation of DNA, and DNAse II deficiency results in an accumulation of undigested DNA fragments within phagocytic cells, capable of activating intracellular nucleic acid sensors.\(^72\)

The recent discovery of LAP has shed some light on this issue. The two ancient systems of phagocytosis and autophagy represent two modes of nutrient acquisition, during abundance and scarcity, respectively. These two evolutionarily conserved pathways converge, however, during the engulfment of pathogens or dead cells.\(^73\) LAP is a process that marries the processes of phagocytosis and autophagy into a fundamentally new concept, allowing us to reinterpret the impact of the autophagy machinery on innate host defense mechanisms (Table 3).

LAP is triggered when an extracellular particle, such as a pathogen, immune complex, or dead cell, is sensed by an extracellular receptor, including Toll-like receptor1/2 (TLR1/2), TLR2/6, TLR4, FcR, and TIM4, and phagocytosed.\(^55,74\)-\(^76\) This engulfment recruits some, but not all, members of the autophagy machinery to the cargo-containing vesicle.\(^55,77\) It is the activity of these autophagic players that facilitates the rapid processing of the cargo via fusion with the lysosomal pathway, which can have a critical role in the degradation of engulfed cargo.\(^77,78\) as well as modulate the resulting immune response.\(^55,75,78\)

Despite sharing common molecular machinery, there currently exist several distinctions that differentiate LAP from canonical autophagy (Figure 2). Originally, LAP and autophagy were distinguished by the structure of the LC3-decorated phagosome (or LAPosome) and the rapidity with which LAP occurs. EM analysis revealed that LAP results in single-membrane structures,\(^77\) as opposed to the double-membrane autophagosomes surrounding autophagic cargo.\(^79\) Whereas LC3-decorated autophagosomes can take hours to form, LC3-II can be detected on LAPosomes in as few as 10 min after phagocytosis, and phosphatidylinositol 3-phosphate (PI(3)P) activity can be seen at the LAPosome within minutes after phagocytosis.\(^55,75,77\)

Recent studies have elucidated the molecular mechanisms governing LAP.\(^78\) Although a majority of the core autophagy components are required for LAP, there exist some critical differences that can distinguish the two processes. Under basal conditions, mTOR inhibits the pre-initiation complex, comprised of FIP200, autophagy-related gene13 (ATG13), and ULK1/2, and hence autophagy. However, the pre-initiation complex is dispensable for LAP.\(^55,76,78\) Furthermore, canonical autophagy requires the ULK1-dependent release of a Beclin1-activating cofactor, Ambra1, from the dynnein motor complex.\(^80\) and the function of WIPI2,\(^81\) whereas LAP does not.\(^78\)

Both LAP and canonical autophagy require the class III PI3K complex, which contains the core components Beclin1,

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### Table 3 Components of the autophagic machinery and their association with inflammatory and autoimmune diseases

| Molecule | Confirmed pathway(s) | Associated Disease(s) | References |
|----------|----------------------|-----------------------|------------|
| NOX2     | LAP                  | CGD, Alzheimer’s disease, Creuzfeldt–Jakob disease | 165–167    |
| Rubicon  | LAP                  | Ataxia                | 168        |
| Beclin1  | Autophagy            | Ovarian cancer, breast cancer, lung cancer, cystic fibrosis, Alzheimer’s disease, RA | 169–174    |
| VPS34    | Autophagy            | Schizophrenia         | 175        |
| UVRAG    | Autophagy            | Stomach cancer, non-segmental vitiligo, colorectal cancer, cardiomyopathy | 85,176–178 |
| ATG5     | Autophagy            | Airway inflammation, SLE/systemic autoimmunity, MS/EAE, RA, Alzheimer’s disease, atherosclerosis | 179–184    |
| ATG16L   | Autophagy            | Crohn’s disease, atherosclerosis | 185,186    |
| ATG7     | Autophagy            | SLE/systemic autoimmunity, MS/EAE, type I diabetes, RA, Alzheimer’s disease, cardiomyopathy | 187–190    |
| ATG4     | Autophagy            | Otoconia              | 191        |
| LC3      | Autophagy            | Nasu-Hakola disease   | 192        |
| LAMP2    | Autophagy            | Danon disease, type II diabetes | 193        |
| ULK1     | Autophagy            | Crohn’s disease       | 194        |
| FIP200   | Autophagy            | Inflammatory skin disorders | 195        |
| p62      | Autophagy            | Huntington’s disease  | 196        |
| EPG5     | Autophagy            | Vici syndrome         | 197        |
| IRG1M    | Autophagy            | Crohn’s disease, MS/EAE | 198        |
| SMURF1   | Autophagy            | Ulcerative colitis    | 199        |
| WDR45    | Autophagy            | Encephalopathy        | 200        |
| Parkin   | Mitophagy            | Parkinson’s disease   | 201        |
| PINK1    | Mitophagy            | Parkinson’s disease   | 202        |

Abbreviations: ATG, autophagy-related gene; CGD, chronic granulomatous disease; EAE, experimental autoimmune encephalitis; LAP, LC3-associated phagocytosis; MS, Multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus

Confirmed activity in autophagy, LAP, and/or mitophagy is indicated.
VPS34, and VPS15. It can, however, differ in its additional composition. ATG14 and UVRAG are mutually exclusive in their association with the class III PI3K complex during autophagy, and silencing of either ATG14 or UVRAG inhibits canonical autophagy. LAP, on the other hand, only requires the activity of the UVRAG-containing class III PI3K complex, whereas ATG14 is dispensible.

Rubicon (RUN domain protein as Beclin 1 interacting and cysteine-rich containing) is a protein that associates constitutively with the UVRAG-containing class III PI3K complex. Rubicon is a negative regulator of autophagy (via its inhibition of VPS34 or by blocking GTPase Rab7 activation), and silencing of Rubicon results in an increase in the number of autophagosomes. During LAP, Rubicon is uniquely associated with Laposomes (but not conventional phagosomes), and Rubicon-deficient cells are completely defective in LAP. Thus, Rubicon is a molecule that is uniquely required for LAP, but dispensable for canonical autophagy.

Studies suggest that the role for Rubicon in LAP is twofold. First, Rubicon promotes the association of the active class III PI3K complex with the Laposome, thereby aiding in the localization of VPS34-mediated PI(3)P at the Laposome.

Figure 2 The processing of engulfed dying cells requires LC3-associated phagocytosis (LAP) and promotes an anti-inflammatory response. Upon engulfment of dying cells, components of the LAP pathway are recruited to dead-cell-containing phagosome (or Laposome). The class III PI3K complex, comprised of Beclin 1, VPS34, UVRAG, and Rubicon, is critical to the sustained and localized production of PI(3)P at the Laposome. PI(3)P serves two roles – the recruitment of the downstream LAP machinery (such as ATG5, ATG12, ATG16L, and ATG7) and stabilization of the NOX2 complex for the production of ROS. Rubicon is also required for the stabilization of the NOX2 complex. Both ROS and PI(3)P are required for successful LC3-II decoration of the Laposome, and LC3-II is required for fusion to the lysosome and maturation of Laposome. The anti-inflammatory effects of efferocytosis are mediated by the activity of lipid and cholesterol sensors, such as ABCA1, LXR, PPARγ, and PGC-1α, leading to the production of IL-10 and TGF-β, whereas pro-inflammatory mediators, such as IL-12, are actively repressed.
In both canonical autophagy and LAP, PI(3)P is required for the recruitment of the downstream ubiquitin-like conjugation systems, the ATG5-12 and LC3-PE conjugation systems. In LAP, Rubicon and PI(3)P have an additional role. Rubicon stabilizes NOX2, the predominant NADPH oxidase in phagocytes, by interacting with its p22phox subunit via its serine-rich domain (aa 567–625), a domain separate from the CCD domain (aa 515–550) responsible for its interaction with Beclin1 and the RUN domain (aa 49–180) responsible for its interaction with VPS34. Moreover, PI(3)P binds and stabilizes the p40phox subunit of NOX2. Collectively, Rubicon promotes the association of the active class III PI3K complex with the LAPosome and the production of PI(3)P. Rubicon and PI(3)P stabilize the active NOX2 complex to promote optimal reactive oxygen species (ROS) production, which is also required for successful LAP. Indeed, NOX2-deficient cells fail to undergo LAp78,91 and scavenging of ROS by antioxidants, such as resveratrol, Tiron, or alpha-tocopherol is also an effective way to inhibit LAP78,86,91. Thus, LAP and canonical autophagy are molecularly distinct processes.13,55,75,76

In addition, LAP and canonical autophagy are functionally distinct as well. There is mounting evidence that LAP is a critical regulator of inflammation in vivo and under physiologically relevant conditions. Not only is LAP critical for the degradation of engulfed organisms, such as intraphagosomal yeast77 or Aspergillus fumigatus, but LAP can have a profound effect on the immune response to the engulfed material. Upon intranasal challenge with A. fumigatus, a TLR2 ligand, LAP-deficient animals fail to efficiently clear the pathogen and display increased levels of pro-inflammatory cytokines both locally (lung) and systemically (serum).74 Thus, many of the autophagic defects associated with control of pathogens could actually be defects in LAP.

LAP can also be triggered in specialized phagocytes, such as the RPE. On a daily basis and regulated by circadian rhythm, RPE cells phagocytose and digest shed POSs, a process crucial for supplying nutrients and O2 to the retina and the metabolism of vitamin A for the visual cycle. However, the receptor(s) that recognize shed POS and trigger LAP remains unknown. What is known is the requirement for LAP in the proper processing of POS and promotion of the visual cycle, a series of biochemical reactions within the RPE and retina that ultimately result in the production of the chromophore 11-cis-retinal (RAL) necessary for the phototransduction signaling cascade. RPE cells deficient for LAP (ATG5, Beclin1), but not canonical autophagy (ULK1, FIP200, ATG13) displayed defective POS degradation, diminished production of 11-cis-retinal, and decreased visual function with age. Thus, LAP functions to support chromophore regeneration through the efficient processing of POS by the RPE.13

LAP is also required for establishing specific signaling compartment and is a critical regulator of the type I interferon response in some cases. In plasmacytoid dendritic cells, LAP is induced by engagement of the FcγR by immune complexes (IC), complexes of self-antigen (such as DNA) and auto-antibodies commonly found in patients with SLE. In cells deficient for LAP, failure to lipidate LC3 on the DNA-IC-containing LAPosome results in a failure to acquire a late-endolysosomal phenotype. Subsequently, these LAP-deficient cells fail to form the specialized interferon regulatory factor 7 (IRF7)-signaling compartment required for TLR9-mediated activation of IRF7, and therefore fail to produce IFN-α. This suggests that LAP could affect the functional immune response elicited by autoantigens and have an important role in autoimmunity.75

Unwanted inflammation and autoimmunity is held in check by the efficient clearance of dying cells every day.55,92 It is the responsibility of the phagocytes to first clear the dying cell from circulation and then instigate an anti-inflammatory response. Phagocytes that have engulfed apoptotic cells have been shown to secrete anti-inflammatory cytokines, such as TGFβ and interleukin-10 (IL-10),54 whereas actively suppressing pro-inflammatory cytokines, such as tumor necrosis factor, IL-1, and IL-12.53 How the phagocyte achieves this feat is of great interest. LAP is triggered during effecrocytosis, and apoptotic, necrotic, and necroptotic cells can engage the PS receptor, TIM4, resulting in a recruitment of the LAP machinery to the dead-cell-containing, single-membrane LAPosome. LAP-deficient macrophages fail to recruit LC3 to the LAPosome, leading to a failure in phagosomal acidification and subsequent corpse degradation. Whereas the paradigm of effecrocytosis is ‘immunologically silent’, LAP-deficient macrophages produce markedly increased levels of IL-1β and IL-6 when fed dying cells, yet produce significantly less anti-inflammatory cytokines, such as IL-10, upon such engulfment.55 LAP is engaged by a variety of receptors and is critical for directing a variety of different immune response, including preventing an unwanted inflammatory response and promoting the formation of the interferon signaling compartment.55,75 Although these functions may appear contradictory, it suggests that the fundamental role of LAP is to shape the appropriate response, and absence of this pathway seems to result in aberrant inflammation and pathogen control.

How the LAP pathway modulates the immune response to apoptotic cells remains to be elucidated, though clues may lie in the mechanisms by which the phagocyte handles the metabolic stress of doubling its content of cellular components. The sensing of one such component, cholesterol, can have a significant effect on the phagocyte’s response to engulfed dead cells and their increase in basal cholesterol efflux activity.94 Members of the peroxisome proliferator-activated receptor γ/δ (PPARγ/δ) and liver X receptor (LXR) families, both important regulators of cellular lipid homeostasis, are activated during effecrocytosis, and results in a positive feedback signal wherein the phagocytic receptors, such as members of the TAM family, are upregulated.55,96 Furthermore, cholesterol efflux machinery, such as 12-transmembrane protein ABCA1 (ATP-binding cassette sub-family A, member 1), is upregulated to accommodate the increase in cholesterol load.92

The non-immunogenic nature of effecrocytosis of apoptotic cells is one of its key characteristics, and cholesterol homeostasis has a critical role in establishing this tolerance.18,22 PPARγ/δ are central players in the polarization of anti-inflammatory (‘M2’) macrophages, and agonists for both PPARγ and LXR have been shown to inhibit inflammatory responses.18,96 Conversely, PPARγ−/− and PPARδ−/− macrophages are defective in effecrocytosis. The dual functions of PPARs and LXRs in both lipid apoptotic cell clearance and
lipid homeostasis suggest the interconnectedness between efferocytosis and metabolism.

Despite all types of dying cells providing excess cholesterol for the engulfing cells, uptake of necrotic cells does not induce enhanced cholesterol efflux in the phagocytes, suggesting that engagement of ligands on apoptotic cells, not extra cholesterol, induces a ‘prophylactic’ cholesterol efflux from phagocytes.

Studies have shown that mere co-culture of macrophages with PtdSer liposomes can induce the cholesterol efflux, anti-inflammatory cytokine production, and suppression of pro-inflammatory genes. These data suggest that metabolic sensors, in conjunction with engagement of ‘eat-me’ signals, such as PtdSer, contribute to the immunological tolerance associated with efferocytosis.

Conclusions: Check Please!

Defects at multiple points in the efferocytosis pathway have been reported to result in unchecked inflammation or autoimmunity, and understanding the mechanisms by which dying cells are effectively cleared can pave the way for therapies that target these processes. Although many studies have examined inflammatory disorders in the context of defective attraction, recognition, and physical engulfment of dying cells, we now recognize that aberrant processing of dead cells, potentially via deviations in LAP, can also result in inflammation. Although systemic disorders, such as SLE, have been long linked to defects in dying cell clearance and the autophagy machinery, more definitive roles for these pathways in ‘localized’ inflammatory diseases, such as ulcerative colitis, atherosclerosis, neurodegeneration, and rheumatoid arthritis should be described. Moreover, the intricate link between inflammation and cancer raises the question of what the role of efferocytosis is during tumor development, metastasis, and chemotherapy-mediated tumor clearance. Although clearance of dying cells is a common occurrence in healthy and diseased cells, recent studies describe the process of entosis, wherein living cells are engulfed by phagocytes. Although some entotic cells can escape from their engulfment unscathed, most are targeted for destruction by LAP. Entosis events are common in human cancers, but their role remains unclear. The mechanisms by which entosis occurs, and its similarity to efferocytosis, implies that the burden that lays before the phagocytic system is a daunting one.

Conflict of Interest

The authors declare no conflict of interest.

1. Green DR. Means To An End: Apoptosis And Other Cell Death Mechanisms. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY, USA, 2011, p 220.
2. Kinchen JM. A model to die for: signaling to apoptotic cell removal in worm, fly and mouse. Apoptosis 2010; 15: 998–1006.
3. Penalozá C, Lin L, Lockshin RA, Zakari Z. Cell death in development: shaping the embryo. Histochim Cell Biol 2006; 126: 149–158.
4. McNew DR, Berger T, Mack TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol 2013; 5: a008656.
5. Obert A, Dillon CP, Weinrich R, McCormick LL, Fitzgerald P, Pop C et al. Catalytic activity of the caspase-8-FP(L) complex inhibits RIPK3-dependent necrosis. Nature 2011; 471: 363–367.
6. Miao EA, Rajan JV, Ademir A. Caspase-1-induced pyroptotic cell death. Immunol Rev 2011; 243: 206–214.
7. Martinez J. Prix fixe: efferocytosis as a four-course meal. Curr Top Microbiol Immunol 2015; 1–36.
39. Park SY, Jung MY, Kim HJ, Lee SJ, Kim SY, Lee BH et al. Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylinerse receptor. Cell Death Differ 2008; 15: 192–201.

40. Hanayama R, Tanaka M, Miwa K, Shinohara A, Inamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature 2002; 417: 182–187.

41. Ishimoto Y, Ohashi K, Mizuno K, Nakano T. Promotion of the uptake of PS immunosomes and apoptotic cells by a product of growth arrest-specific gene, gas6. J Biochem 2000; 127: 411–417.

42. Zizzo G, Hilliard BA, Monestier M, Cohen PL. Efficient clearance of early apoptotic cells by CD47 as a marker of self on red blood cells. Science 2006; 312: 1582–1586.

43. Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the clearance of dead cells. Methods Find Exp Clin Pharmacol 2008; 30: 3643–3654.

44. Bergsma T, Prik SL, Cookson BT. Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 2009; 7: 99–109.

45. Hanayama R, Tanaka M, Miwa K, Shinohara A, Inamatsu A, Nagata S. Constitutive expression of phosphatidylinerse on viable cells. Proc Natl Acad Sci USA 2011; 108: 19246–19251.

46. van den Eijnde SM, van den Hoff MJ, Retelingsperger CP, van Heerde WL, Hentling ME, Vermeer-Kees C et al. Transient expression of phosphatidylinerse at cell-cell contact areas is required for myotube formation. J Cell Sci 2001; 114(Pt 20): 3631–3643.

47. Oldenberg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science 2000; 288: 2051–2054.

48. Elward K, Griffiths M, Mizuno M, Harris CL, Neal JW, Morgan BP et al. CD46 plays a key role in tailoring innate immune recognition of apoptotic and necrotic cells. J Biol Chem 2002; 277: 1416–1428.

49. Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science 2000; 288: 2051–2054.

50. Elward K, Griffiths M, Mizuno M, Harris CL, Neal JW, Morgan BP et al. CD46 plays a key role in tailoring innate immune recognition of apoptotic and necrotic cells. J Biol Chem 2002; 277: 1416–1428.

51. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R et al. Transient expression of phosphatidylinerse at cell-cell contact areas is required for myotube formation. J Cell Sci 2001; 114(Pt 20): 3631–3643.

52. Fadok VA, Bratlton DL, Gultine L, Herson PM. Differential effects of apoptotic versus lysed cells on macrophage production of cytokines: role of proteases. J Immunol 2001; 166: 6847–6854.

53. Martinez J, Almendrillo J, Obert A, Ness R, Dillon CP, Fitzgerald P et al. Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. Proc Natl Acad Sci USA 2011; 108: 17398–17401.

54. Camins A, Palusz M, Silvestre JS. Apoptotic mechanisms involved in neurodegenerative diseases: experimental and therapeutic approaches. Methods Find Exp Clin Pharmacol 2008; 30: 43–65.

55. Lee BY, Kim HJ, Lee CB, Kwon IC, Kim IS. Molecular targeting of atherosclerotic plaques by a stabilin-2-specific peptide ligand. Proc Natl Acad Sci USA 2012; 109: 15338–15343.

56. Martinez J, Verbist K, Wang R, Green DR. The relationship between metabolism and the autophagy machinery during the innate immune response. Cell Metab 2013; 17: 895–900.

57. Lee GY, Kim JH, Oh GT, Lee BH, Kwon IC, Kim IS. Molecular targeting of atherosclerotic plaques by a stabilin-2-specific peptide ligand. Proc Natl Acad Sci USA 2012; 109: 15338–15343.

58. Sokolowski JD, Mandell JW. Phagocytic clearance in neurodegeneration. J Exp Med 2011; 208: 3508–3520.

59. Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletter S, Gingrich S et al. Molecular characterization of LC3-associated phagocytosis (LAP) reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 2015; 17: 893–906.

60. Henaut J, Martinez J, Riggs JM, Tian J, Mehta P, Clarke L et al. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. Immunity 2012; 37: 388–397.

61. Florey O, Kim SE, Sandoval CP, Haynes CM, Overholtzer M. Autophagy machinery mediates phagosome-membrane processing and entitic cell death by targeting single membranes. Nat Cell Biol 2011; 13: 1335–1343.

62. Sanjuan MA, Dillon CP, Talt SW, Moshiach S, Dorsey F, Connell S et al. Toll-like receptor signaling in macrophages links the autophagy pathway to phagocytosis. Nature 2007; 450: 940–944.

63. Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletter S, Gingrich S et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 2015; 17: 893–906.

64. Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-lysophosphatidylinositol-3-kinase complex. Nat Cell Biol 2009; 11: 486–476.

65. Song Z, An L, Ye Y, Wu J, Zou Y, He L et al. Essential role for UVAG in autophagy and maintenance of cardiac function. Cardiovasc Res 2014; 101: 48–56.

66. Matsunaga K, Saitoh T, Tabata K, Omori H, Sato T, Kurotori N et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol 2009; 11: 385–396.

67. Sun Q, Westphal W, Wong KN, Yan Z, Zhang Q. Rubicon controls endothose maturation as a prosurvival effector. Proc Natl Acad Sci USA 2010; 107: 13393–13398.

68. Yang CS, Lee JS, Rodgers M, Min CK, Lee JY, Kim HJ et al. Autophagy protein Rubicon mediates phagocytic NADPH oxidase activation in response to microbial infection or TLR stimulation. Cell Host Microbe 2012; 11: 264–276.

69. Sun Q, Zhang J, Fan W, Wong KN, Ding X, Chen S et al. The RN domain of rubicon is important for Nps3b binding, lipid kinase inhibition, and autophagy suppression. J Biol Chem 2011; 286: 185–191.

70. Ueyama T, Nakakita J, Nakamura T, Kobayashi T, Kobayashi T, Son J et al. Cooperation of p40(phox) with p47(phox) for Nox2-based NADPH oxidase activation during Fcgamma receptor (FcgammAR)-mediated phagocytosis: mechanism for acquisition of p40(phox) and maintenance of cardiac function. J Biol Chem 2011; 286: 40693–40705.

71. Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, Magalhaes MA et al. Activation of antibacterial oxidase by NADPH oxidase in response to microbial infection or TLR stimulation. Cell Host Microbe 2012; 11: 264–276.

72. Kim S, Elkon IB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity 2004; 21: 643–653.

73. A-Gonzalez N, Benisnger SJ, Hong C, Beevor S, Bradley MN, Zelker N et al. Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. Immunity 2009; 31: 245–258.

74. Rosser T, Menendez-Gutierrez MP, Lefevrea MI, Alameda D, Nunez V, Lazar MA et al. Autimmune kidney disease and impaired engulfment of apoptotic cells in mice with macrophage peroxisome proliferator-activated receptor gamma or retinoid X receptor alpha deficiency. J Immunol 2011; 180: 621–631.

75. Mukundan L, Odegaard JI, Morel CR, Heredia JE, Mwangi JW, Ricardo-Gonzalez RR et al. PPAR-delta senses and orchestrates clearance of apoptotic cells to promote tolerance. Nat Med 2009; 15: 1266–1272.
121. Bolick DT, Skaflen MD, Johnson LE, Kwon SC, Howatt D, Daugherty A et al. Immunogenic and tolerogenic cell death required for the clearance of dying cells. Nature Immunology 2015; 16: 1109–1116.

122. Zhub D, Li C, Swanson AM, Villaboa RM, Guo J, Zhang Z et al. BAX regulates spatial learning and synaptic plasticity in the hippocampus. J Clin Invest 2015; 125: 4047–4058.

123. Sutcliffe D, Bouey DM, Gobets J, van der Zypen M, van der Flier WM, van der Heijden M et al. A unified model of mammalian BCL-2 protein family interactions at the mitochondrial membrane. Mol Cell 2011; 44: 517–531.

124. Boatright KM, Renatus M, Scott FL, Sparrando S, Shin H, Pedersen IM et al. Antigen-specific CD8+ T cell-mediated clearance of tumour cells by a novel aromatic amino acid. J Immunol 2008; 180: 6106–6113.

125. McIntyre JI, Umetsu SE, Aiello O, Potter M, Kuchroo VK, Bashir GS et al. Identification of TCR (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat Immunol 2001; 2: 1109–1116.

126. Flavell RA, Skaflén MD, Johnson LE, Kwon SC, Howatt D, Daugherty A et al. Immunogenic and tolerogenic cell death required for the clearance of dying cells. Nature Immunology 2015; 16: 1109–1116.
154. Ranimani AC, Tili SJ, Garcia C, Capel F, Gotth V, Balabanian K et al. The CX3C chemokine fractalkine in allergic asthma and rhinitis. J Allergy Clin Immunol 2003; 112: 1139–1146.

155. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Mol Med (Berl) 2009; 87: 1323–1337.

156. Baker OJ, Camden JM, Rome DE, Seye CI, Weisman GA. P2Y2 nucleotide receptor promoter region of PIK3C3 in schizophrenia. Neurosci Lett 2010; 475: 287–293.

157. Rimaniol AC, Till SJ, Garcia G, Capel F, Godot V, Balabanian K et al. CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Nat Med 2006; 12: 445–452.

158. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA et al. Genetic variants in the autophagy gene ULK1 and risk of Crohn’s disease. Inflamm Bowel Dis 2014; 20: 1397–1403.

159. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Allergy Clin Immunol 2003; 112: 1139–1146.

160. Tang R, Zhao X, Fang C, Tang W, Huang K, Wang L. Migration of CX3CR1-positive T cells expressing type 1 cytokines and cytotoxic molecules into the proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Nat Med 2006; 12: 445–452.

161. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y et al. Defective autophagy-related gene 6 levels are a reliable biomarker of disease activity in systemic lupus erythematosus. J Biol Chem 2014; 289: 1063–1073.

162. Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS et al. The clearance of dying cells enhances lymphocyte adherence to a human submandibular gland cell line. Mol Immunol 2010; 47: 1139–1149.

163. Yanagita M, Ishimoto Y, Arai H, Nagai K, Ito T, Nakano T et al. Essential role of Ga6 for gliomerular inflammation in nephrotic nephritis. J Clin Invest 2002; 110: 239–246.

164. Kim HA, Nam JY, Jeon JY, An JM, Jung JY, Bae CB et al. Serine growth arrest-specific protein 6 levels are a reliable biomarker of disease activity in systemic lupus erythematosus. J Clin Immunol 2013; 33: 143–150.

165. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T et al. Activation of NADPH oxidase in Alzheimer’s disease brains. Biochim Biophys Acta 2003; 1629: 253–262.

166. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T et al. Activation of NADPH oxidase in Alzheimer’s disease brains. Biochim Biophys Acta 2003; 1629: 253–262.

167. Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. J Exp Med 2003; 198: 729–739.

168. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA et al. Genetic variants in the autophagy gene ULK1 and risk of Crohn’s disease. Inflamm Bowel Dis 2014; 20: 1397–1403.

169. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Allergy Clin Immunol 2003; 112: 1139–1146.

170. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H et al. Defective autophagy-related protein 6 levels are a reliable biomarker of disease activity in systemic lupus erythematosus. J Biol Chem 2014; 289: 1063–1073.

171. Hofmann MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y et al. Defective autophagy-related protein 6 levels are a reliable biomarker of disease activity in systemic lupus erythematosus. J Biol Chem 2014; 289: 1063–1073.

172. Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS et al. The clearance of dying cells enhances lymphocyte adherence to a human submandibular gland cell line. Mol Immunol 2010; 47: 1139–1149.

173. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA et al. The role of the NADPH oxidase NOX2 in microglial cell survival and death: implications for the inflammatory response in Alzheimer’s disease. J Neurosci 2003; 23: 7259–7268.

174. Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA et al. The role of the NADPH oxidase NOX2 in microglial cell survival and death: implications for the inflammatory response in Alzheimer’s disease. J Neurosci 2003; 23: 7259–7268.

175. Tang R, Zhao X, Fang C, Tang W, Huang K, Wang L et al. Investigation of variants in the autophagy gene ULK1 and risk of Crohn’s disease. Inflamm Bowel Dis 2014; 20: 1397–1403.

176. Shimohama S, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T et al. Activation of NADPH oxidase in Alzheimer’s disease brains. Biochim Biophys Acta 2003; 1629: 253–262.

177. Jeong TJ, Shin MK, Umh YK, Kim HJ, Chung JH, Lee MH. Association of UVRAG polymorphisms with susceptibility to non-segmental vitiligo in a Korean sample. Exp Dermatol 2010; 19: 323–329.

178. Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS et al. The clearance of dying cells enhances lymphocyte adherence to a human submandibular gland cell line. Mol Immunol 2010; 47: 1139–1149.

179. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Allergy Clin Immunol 2003; 112: 1139–1146.

180. Zhao XJ, Lu XL, Lv JC, Yang HZ, Qin LX, Zhao MH et al. Genetic association of PRDM1-ATG5 intergenic region and autophagy with systemic lupus erythematosus in a Chinese population. Ann Rheum Dis 2011; 70: 1330–1337.

181. Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. J Exp Med 2011; 208: 2625–2632.

182. Tani Y, Bustos V, Flajolet M, Greengard P. A small-molecule enhancer of autophagy decreases levels of Abeta and APP-CTF via Atg5-dependent autophagy pathway. FASEB J 2011; 25: 1934–1942.

183. Han JW, Zheng HF, Cui Y, Sun LD, Ye DG, Hu Z et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet 2009; 41: 1234–1237.

184. Liao X, Slumer JC, Wang Y, Subramanian M, Brown K, Pattison JS et al. Macrophage autophagy plays a protective role in advanced atherosclerosis. Cell Metab 2012; 15: 465–473.

185. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lenner J et al. The key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 2008; 456: 259–263.

186. Raychaudhuri S, Thomson BP, Remmers EF, Eeke S, Hinks A, Guducu C et al. Genetic variants at CD208, PRDM1 and CD23/D2S8 are associated with rheumatoid arthritis risk. Nat Genet 2009; 41: 1313–1318.

187. Bhuyan MS, Pattison JS, Osinska H, James G, Julick J, McLendon PM et al. Enhanced autophagy ameliorates cardiac proteinopathy. J Clin Invest 2013; 123: 5288–5297.

188. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Allergy Clin Immunol 2003; 112: 1139–1146.

189. Relvas LJ, Makhoul M, Dewispelaere R, Caspers L, Communi D, Boeynaems JM et al. Heat-shock proteins can promote as well as regulate autoimmunity. J Allergy Clin Immunol 2003; 112: 1139–1146.