Chapter

Efferocytosis: An Interface between Apoptosis and Pathophysiology

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Abstract

Several cell death modes, each with a unique feature and mode of inducing cell death have been established. Cell death occurring under physiological conditions is primarily caused by apoptosis, which is a non-inflammatory or silent process, whereas necroptosis or pyroptosis is triggered by pathogen invasion, which stimulates the immune system and induces inflammation. In physiology, clearing dead cells and associated cellular debris is necessary since billions of cells die during mammalian embryogenesis and every day in adult organisms. For degradation, dead cells produced by apoptosis are quickly engulfed by macrophages. This chapter will present a description of the phagocytosis of dead and dying cells, by a process known as efferocytosis. Macrophages and, to a lesser degree, other 'professional' phagocytes (such as monocytes and dendritic cells) and 'non-professional' phagocytes, such as epithelial cells, conduct efferocytosis. Recent discoveries have shed light on this mechanism and how it works to preserve homeostasis of tissue, repair of tissue and health of the organism. Caspases are a large family of proteases of cysteine acting in cascades. A cascade leading to activation of caspase 3 mediates apoptosis and is responsible for killing cells, hiring macrophages, and presenting a "eat me" signal(s). If macrophages do not effectively engulf apoptotic cells, they undergo secondary necrosis and release intracellular materials that reflect a molecular pattern associated with injury, which can lead to autoimmune diseases. Here, the processes of efferocytosis are illustrated and the pathophysiological effects that which occur when this phase is abrogated are highlighted.

Keywords: apoptosis, efferocytosis, macrophages, caspases, pyroptosis, necroptosis

1. Introduction

In multicellular species, cell death and the efficient clearance of dying cells are fundamental processes that preserve homeostasis. Cells engage in their demise in virtually all physiological and most pathological scenarios through a controlled cascade of signaling events ('regulated' cell death) [1] Damaged or redundant cells die in a regulated manner and are replaced by new cells derived from progenitors of stem cells [2]. Cell death is essential for growth; during mammalian embryogenesis and development, billions of cells are destroyed in order to form new structures and sustain organ function [3, 4]. When addressing pathological events, including tissue damage and infections, significant numbers of cells often die. Cell death must

be carefully controlled; extensive damage caused by heat, mechanical compression or osmotic pressure, for example, can cause necrosis of cells, release their intracellular content to the surrounding environment, and contribute to the activation of inflammatory immune pathways that can harm healthy cells and tissues surrounding them. In both homeostasis and disease, removal of cellular corpses is necessary. The phagocyte engulfment of dead cells, a multistep mechanism known as efferocytosis, enables cellular components to be recycled by multicellular organisms. Autoimmune diseases and other diseases may occur when the disposal of cell corpses is faulty. While the degradation and recycling of the mass of a cell are typical features in the clearance of any dead cell, certain cell clearance characteristics are specific to a particular mode of cell death. Depending on their mode of death, dying cells can expose and secrete signals that attract phagocytes, favor their swallowing, or encourage a return to tissue homeostasis. Via regulation of macrophage function following efferocytosis, different forms of cell death may also confer pro-inflammatory or anti-inflammatory signals. There are several cases in which uncoupled phenomena are cell death and corpse disposal, meaning cell death does not follow disposal immediately. The shedding of dead intestinal epithelial cells from the tip of the villus to the intestinal lumen 5 is one such example. Conversely, cells engulf and destroy healthy neighbouring cells in the course of entosis. Although the responsible mechanisms are usually different from those for the removal of dead cells, [5–11] shared aspects have been identified. Nonetheless, dying cells are easily cleared by efferocytosis in the vast majority of cases, and such uncoupled events are not further considered herein. Here we summarise our current understanding of the mechanisms of efferocytosis and how the physiology of the organism is affected by efferocytosis, including inflammation and adaptive immune response results. Every day, several hundred billion cells die and are replaced by newly formed cells. Senescent neutrophils die and are then phagocytized in the bone marrow, liver, and spleen by macrophages [7, 8], while senescent red blood cells are recognized and phagocytized by spleen and liver macrophages [7, 8]. In the small intestine, senescent enterocytes are shed into the intestinal lumen [11]. Cells infected with bacteria or viruses undergo autonomous necrosis of the cells or are destroyed by the immune system. Macrophages as a whole do not recognize these cells, and necrotic cells release materials that may activate the immune system. Inflammation caused by a bacterial or viral infection requires significant white blood cell development, and these cells rapidly die when the infection ceases. Additionally, by inhibiting the synthesis of purines, DNA, or RNA or by cross-linking or intercalating DNA, various anticancer drugs destroy tumour cells.

2. Programmed cell death

Originally, programmed cell death in the sense of insect development was described by Lockshin and Williams [2]. Subsequently, Kerr et al. [12] noted two morphologically distinct forms of cell death in humans, apoptosis and necrosis, through ultrastructural study. The cells swell in necrosis, with the rupture of plasma membranes and release of cellular components; the cells shrink with integral yet ruffling plasma membranes in apoptosis, and nuclei are condensed and fragmented. To avoid the release of intracellular elements, apoptotic cells are rapidly phagocytized by macrophages. This mechanism prevents inflammatory factors from being released and is thus called the death of clean cells. “Programmed cell death” and “apoptosis” are also used synonymously, because apoptosis occurs in developing embryos or in cells that die under physiological conditions. The cell death occurring during inflammation or infection with a necrotic morphology was also found

to be programmed or regulated by gene products and was classified as necroptosis and pyroptosis [13]. In addition, non-apoptotic cell death was observed during the development of *Caenorhabditis elegans* [14] and *Drosophila metamorphosis* [15], suggesting that cell death can occur through a non-apoptotic mechanism in animal development. Programmed cell death should therefore not be used as a synonym for apoptosis; it should, as originally described, be reserved for the cell death that takes place in animal development [2].

2.1 Apoptosis

In animal growth, apoptosis plays a significant role. For instance, apoptosis removes interdigital cells, non-functional nerve cells, and activated lymphocytes. Apoptosis also mediates the involution of mammary glands. Apoptosis, does not play a significant role in clearing senescent cells (red blood cells, and intestinal enterocytes). Specific sets of caspases that function in cascades mediate apoptosis, at the end of which caspase 3 or 7 is responsible for killing the cells. In a mechanism called efferocytosis, apoptotic cells are engulfed by macrophages [16]. It is difficult to find free apoptotic cells *in vivo*, even in tissues where large numbers of cells undergo apoptosis, because efferocytosis is effective and rapid [17]. Apoptosis therefore requires pathways not just for destroying cells, but also for recruiting Macrophages ("find me") [18, 19] and providing the macrophages with a signal ("eat me") for cell engulfment [20, 21].

3. Recognition of dying cells

In early studies, cell shrinkage prior to cell death was associated with a process of non-inflammatory cell clearance, while inflammatory response is associated with cell swelling prior to death [12–14] (**Figure 1**). These morphologies were associated with apoptosis and necrosis processes. In recent decades, more molecularly diverse controlled cell death programs have been identified [2], with each type having a major impact on the biological consequences of cell death. Both apoptotic and non-apoptotic dying cells demonstrate and release molecular signals in order to signal phagocytes and direct subsequent phagocytic and immune responses.

4. Efferocytosis

The term "efferocytosis" is derived from the Latin word "efferre" meaning "to take to the grave," which describes the process of programmed cell removal (PrCR). In cell biology, efferocytosis is characterized as the swallowing and decomposition of apoptotic cells by phagocytes. The noteworthy point of efferocytosis is that this mechanism includes the production of anti-inflammatory cytokines and the inhibition of pro-inflammatory cytokines. This means that efferocytosis takes place in an immunologically silent state that mechanistically differs from the phagocytosis of pathogens and other opsonized particles [20] in which pro-inflammatory cytokines are produced. Recent evidence has shown that many types of cells have the ability to clear their neighboring dying cells, including both professional and non-professional phagocytes, although it has long been believed that apoptotic cells can only be cleared by efferocytosis by specialized cells. Professional phagocytes include macrophages and immature dendritic cells, while non-professional phagocytes are involved in epithelial cells, endothelial cells, and fibroblasts. The stages in which apoptotic particles are extracted by professional and non-professional phagocytes are the

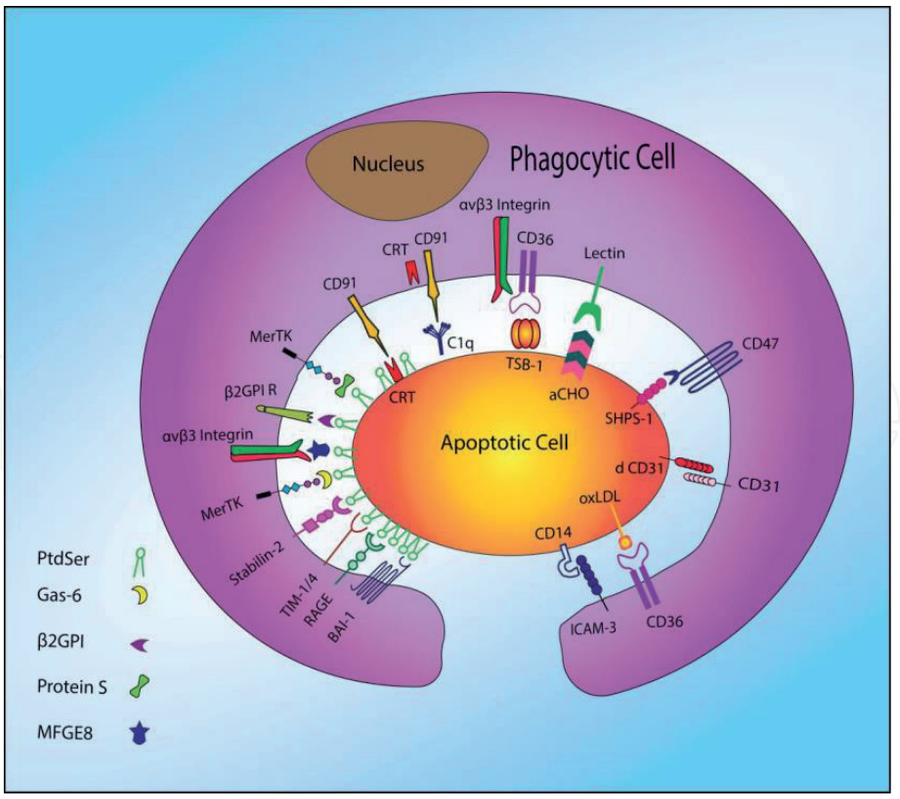

Figure 1.
Recognition of apoptotic cells by phagocytes (The Engulfment Synapse). altered carbohydrate (aCHO), β2-glycoprotein I receptor (β2GPIR), brain-specific angiogenesis inhibitor (BAI-1), complement protein C1q (C1q), calreticulin (CRT), growth arrest-specific 6 (Gas6), intercellular adhesion molecule 3 (ICAM-3), Mer tyrosine kynase (MerTK), milk fat globule-EGF factor 8 protein (MFGE8), oxidized low-density lipoprotein (oxLDL), phosphatidyl serin (PtdSer), receptor for advanced glycation end products (RAGE), Src homology 2 domain-bearing protein tyrosine phosphatase substrate-1 (SHPS-1), T-cell immunoglobulin mucin receptor (TIM), thrombospondin-1 (TSB-1).

same, although various kinetics have been recorded. In fact, the process of efferocytosis is carried out at greater speed and capacity by trained phagocytes [22, 23].

4.1 Find-me signals

Signals produced by apoptotic cells usually involve two types: soluble molecules and complicated extracellular vesicles. Nucleotides such as adenosine triphosphate (ATP) and uridine triphosphate are among the most widely recognized find-me signals (UTP). In the environment, such nucleotides are released by apoptotic cells through the pannexin-1 channel. They bind purinoreceptor 2 (P2Y2) to the surface of the phagocytes, thus serving as short-range chemo attractants [22–27]. Recent studies have shown that certain mediators of oxidative stress can serve as find-me signals by activating monocyte recruitment towards suffering cells at risk of death. The release of oxidative stress mediators may be a physiological mechanism for the precautionary use of phagocytes before the onset of cell death, but it may also promote the pathogenesis of some conditions [1, 9, 28–31]. Lysophosphatidylcholine (LPC) is a lipid mediator formed and released from apoptotic cells by the calcium-independent isoform of phospholipase A22 (iPLA2). LPC plays a part in the recruitment of macrophages by reacting with the G2 accumulation (G2A) receptor. G2A is a G-protein-coupled (GPCR) receptor that plays a role in the regulation of cell cycles, oncogenesis, immunity, and proliferation. G2A is found in macrophages, dendritic cells, neutrophils, mast cells, T lymphocytes, and B lymphocytes. Although awareness of this receptor has so far been restricted, its most important

role as an LPC receptor is probably to enable the development of monocyte, neutrophil and lymphocyte recruitment chemo attractants.

Fractalkine (CX3CL1) is a find-me signal which is secreted from apoptotic human B cells in extracellular vesicles. Fractalkine acts as a chemokine which facilitates its recruitment into apoptotic cells by binding to its receptor (CX3CR1) on the surface of the phagocyte 13. Fractalkine also increases the expression of MFGE8, which plays a role as a bridging molecule, in phase 13 of efferocytosis. In addition, apoptotic cells can cause the release of a number of cytokines and chemokines, including MCP-1 and IL-8, involved in the recruitment of monocytes and Tam-Horsfall protein 1 (THP-1) neutrophils, by binding to Fas/CD95 [32].

4.2 Eat-me signals

By binding to their receptors on the surface of phagocytes, eat-me molecules produce intracellular signal cascades, rearranging the cytoskeleton and contributing to swelling. Eat-me signals are usually divided into two groups [33–35]. The first group consists of new emerging molecules, such as phosphatidylserine (PtdSer) or annexin I, on the surface of apoptotic cells. The second category consists of existing molecules changed on the surface of the apoptotic cells [e.g., conversion of the intercellular adhesion molecule (ICAM)-3 to CD31] or various modifications in the plasma membrane components (e.g., loss of plasma membrane asymmetry and increased external negative charges) (**Figure 2**). Among the new evolving molecules on the surface of apoptotic cells, PtdSer is the most popular and well-known eat-me signal. PtdSer is actually located naturally on the inner surface of the plasma

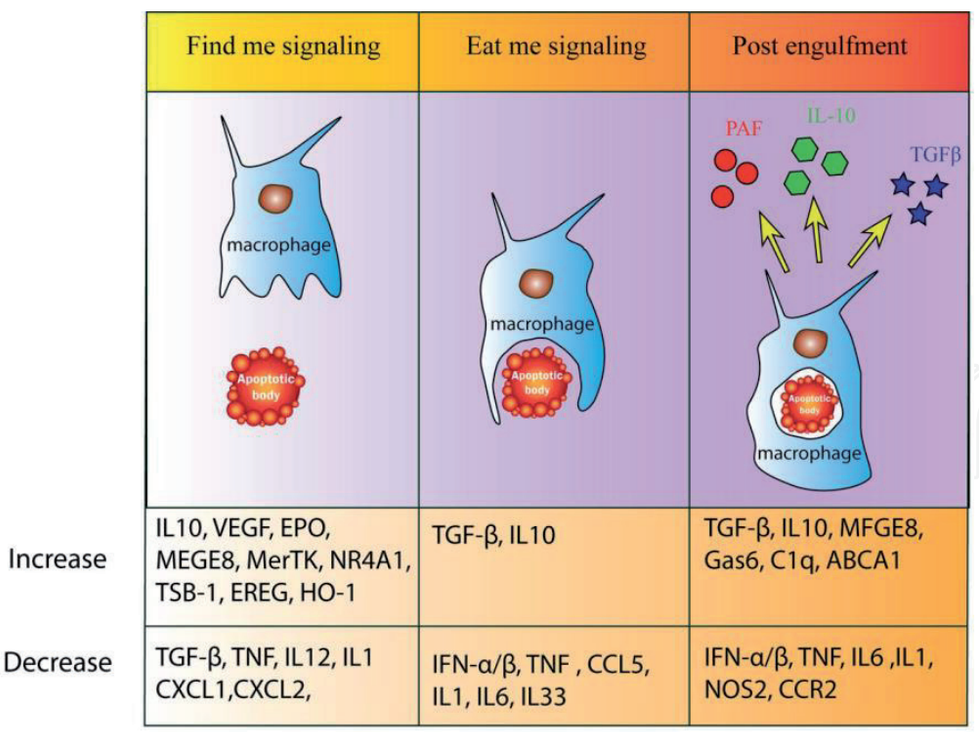

Figure 2.
Apoptotic cell clearance-a multi-stage process. ATP-binding cassette transporter (ABCA1), complement component 1q (C1q), chemokine C-C motif ligand 5 (CCL5), C-C chemokine receptor type 2 (CCR2), chemokine C-X-C motif ligand (CXCL), erythropoietin (EPO), epiregulin (EREG), growth arrest-specific 6 (Gas6), heme oxygenase-1 (HO-1), interferon (IFN), interleukin (IL), Mer tyrosine kinase (MerTK), milk fat globule-EGF factor 8 protein (MFGE8), nitric oxide synthase 2 (NOS2), nuclear receptor subfamily 4 group A (Nr4a1), thrombospondin-1 (TSB-1), transforming growth factor β (TGF-β), tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF).

membrane, but is transmitted to apoptotic cells on the outer surface of the plasma membrane. Flippases are responsible for preserving an asymmetric distribution of phospholipids within the cell membrane in non-apoptotic cells. However, when the apoptosis message is given and caspases in apoptotic cells are activated, flippases become cleaved and inactive. Caspases also simultaneously activate scramblases in apoptotic cells and, as a result, PtdSer rapidly migrates to the outer surface of the plasma membrane [36–40].

PtdSer can bind directly to various surface receptors of phagocytes, including T-cell immunoglobulin mucin (TIM)-1, TIM-4, BAI-1, stabilin-2, and advanced glycation end-product receptors (RAGE). Among them, by binding to PtdSer, TIM-4 is crucial in the tethering of apoptotic cells. Of note, among all phagocytes, some of these receptors do not have the Same distribution [41–46]. Therefore, in the clearance of specific apoptotic cells, each phagocyte with a particular set of these receptors plays a certain function [32, 37–40, 47].

4.3 Bridging molecules

Two domains of receptor-binding have bridging molecules. The prey-binding domain of phagocytosis (PPBD) can, on the one hand, be connected to the surface of apoptotic cells. On the other side, the receptor-binding domain binds to phagocytes. [43, 48–53] A bridge between apoptotic cells and phagocytic cells is thus formed. PtdSer can be indirectly connected to phagocyte receptors by bridging molecules, in addition to its ability to bind directly to receptors on the phagocyte surface. MFGE8 is a bridging molecule which, on the one hand, binds to PtdSer on the surface of apoptotic cells and, on the other, binds to alpha-3/alpha-5 integrins, thus acting as a bridge between apoptotic cells and macrophages in order to facilitate the tethering phase [44, 54–56]. After binding to membrane integrins, it generates signals within macrophages, by recruiting the CRKII-DOCK180-ELMO (**Figure 3**) complex and activating Rac1. These signaling pathways eventually result in a rearrangement of the cytoskeleton and thus, enabling macrophages in the removal of apoptotic cells***. In addition, MFGE8 induces the release of anti-inflammatory cytokines. The anti-inflammatory action of MFGE8 could take place in two ways: directly, through phagocytic capacity development, and indirectly, through the modulation of lipopolysaccharide-activated intracellular signals (LPS) [57–61]. This subset of macrophages promotes tissue repair and angiogenesis through the production of vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF), which is usually found at the stage of tumor promotion. Annexin I (AnxI) is a bridging molecule that is a normal intracellular protein but is transferred to the outer surface of the PtdSer [62] plaque-containing cell membrane during apoptosis.

4.4 Other eat-me signals

While phosphatidylserine is a powerful and well-characterized eat-me signal, other signals may play a role in the identification and swallowing of dying cells [63]. Exposed LPC can bind to IgM on the plasma membrane of dying cells, which in turn binds to phagocyte Fc receptors, such as macrophages [15]. Therefore, LPC continues to serve as a find-me signal as well as an eat-me signal. Proteins present in the endoplasmic reticulum lumen, such as calreticulin, may be exposed to the plasma membrane of dying cells and, in the absence of no-eat-me signals, may serve as an intake-me signal (**Figure 2**). In coordination with complement C1q and mannose-binding lectin (MBL) [16, 17] phagocyte calreticulin is recognized by LDL receptor-related protein 1 (LRP1), also referred to as CD49 [64].

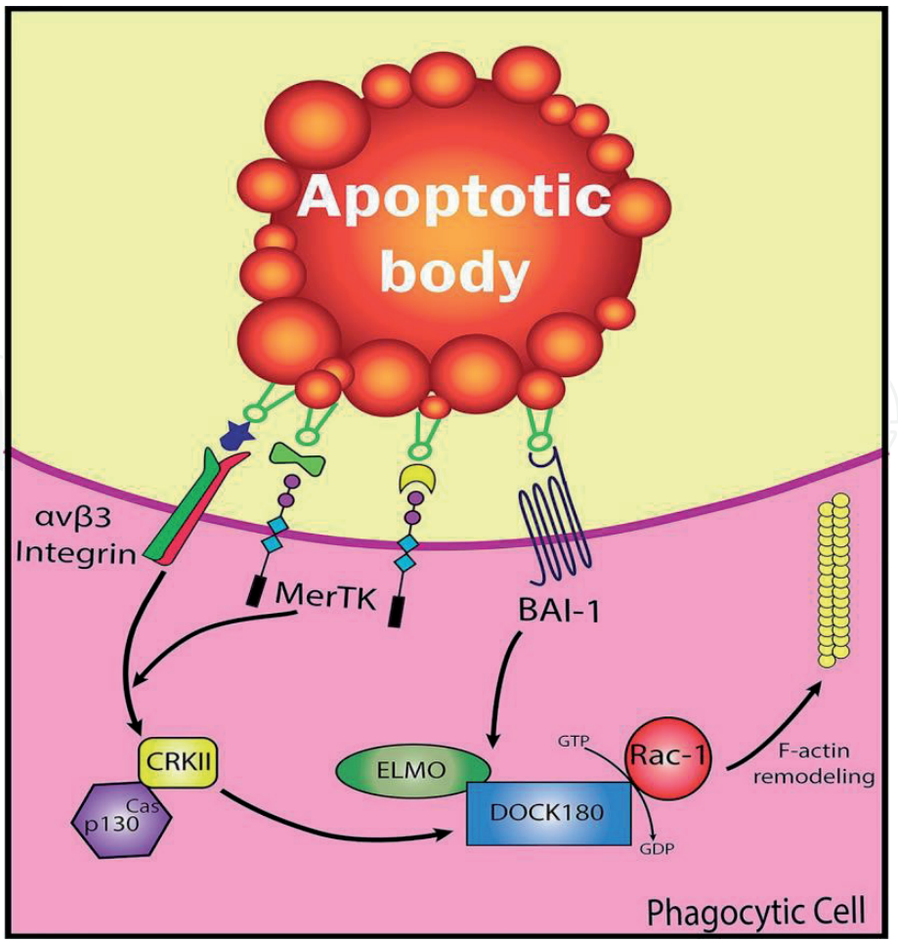

Figure 3.
Activation of the CRKII-DOCK180-ELMO complex within phagocytes. MFGE8-opsonised apoptotic cells are captured by avβ integrins in macrophages; then, the recruitment of the ELMO– DOCK180 complex is induced and finally, by activation of Rac1, cytoskeletal rearrangement occurs for phagocytosis. Brain-specific angiogenesis inhibitor (BAI-1), cellular apoptosis susceptibility protein (Cas), guanosine diphosphate (GDP), guanosine triphosphate (GTP).

4.5 Don't-eat-me signals

Macrophages and other phagocytes are also able to detect live and viable cells through don't-eat-me signals, in addition to their ability to distinguish apoptotic cells from pathogens through eat-me signals. The most important ligand that plays this role is CD47 [65–68]. The signal regulatory protein alpha (SIRP alpha) is the receptor on the surface of macrophages for this protein, the activation of which prevents swallowing even when there is PtdSer [69, 70]. Instead, on the surface of apoptotic cells, CD47 expression is suppressed so that their clearance can be correctly achieved. In transducing don't-eat-me signals, CD31 is another important ligand. Although this protein is not as well-known as CD47, its inhibitory effect on phagocytes [69, 71–73] has been demonstrated by several studies. Another protein that has a critical role in the transduction of a don't-eat-me signal is CD300a. The CD300a-mediated recognition of PtdSer and phosphatidylethanolamine inhibits efferocytosis, preventing swallowing at stage 22.

5. Mechanisms of engulfment

Efferocytosis is a closely regulated process that involves the synchronized swallowing of dead and dying cells, maturation of the phagosome, and then the

breakdown of phagolysosomal material. Each stage is regulated by molecular mechanisms that allow the engulfed cell to quickly break down and recover the engulfing phagocyte.

5.1 Uptake of dying cells

If a dying cell is recognized by the phagocyte, swallowing of the cell corpse requires rapid re-organization and synthesis of the plasma membrane to enable the dead cell to be effectively phagocytic internalized. Phagocyte motility and environmental sampling are allowed by a complex mesh of cortical actin fibres lying under the plasma membrane. The phagocyte initiates actin remodeling upon identification of a dying cell, which enables invagination and localised extravagation of the plasma membrane and, eventually, phagosome creation. Depending on the receptor involved, the signaling mechanisms that bridge receptor ligation to activation of actin remodeling and related pathways vary, but usually include coordinated kinase activation (such as those from the SRK, SYK, and protein kinase C families) and phosphatase inactivation, like SHP1, processes that have been well reviewed [20]. Two primary pathways leading to the reorganization of actin occur during efferocytosis and both converge on a central regulator, the small GTPase RAC1 family of RHO. The activation of RAC1 is mediated by LRP1 and the adapter protein GULP96 in the first method, but the exact mechanism of how LRP1 and GULP activate RAC1 is not understood. The second RAC1 activation mechanism is based on the DOCK180 guanine nucleotide exchange factor (GEF) and the phagocytic regulatory protein engulfment and protein motility of cells (ELMO). Another GEF, called 'TRIO', loads GTP onto the small GTPase RHOG following receptor ligation by the dying cell, leading to ELMO [20, 74–76] recruitment. ELMO is then able to interact with DOCK180's SH3 domain [77]. The DOCK180-ELMO complex, together, serves as a GEF for RAC1, leading to activation of RAC1. By activating nucleation-promoting factors of the WASP family, SCAR and WAVE, which recruit the ARP2/3 complex and work together to build an actin nucleation centre, RAC1 is subsequently able to guide localized actin polymerization required to coat or grasp the cargo once triggered through either system [70, 78–80]. The ARP2/3 complex binds existing actin filaments in addition to forming a nucleation center for de novo actin polymerization, enabling new actin synthesis while retaining actin networking and branching, processes that are crucial for the formation of phagosomes [81–86]. Although actin polymerization is a critical component of the creation of phagosomes and the successful capture of the dead cell, actin depolymerization is of equal importance for the plasma membrane splitting of the phagosome. Phagosome sealing processes are highly dependent on different phosphoinositides, including phosphatidylinositol 3,4,5-trisphosphate, which activates GTPase-activating proteins of the RHO family, resulting in the deactivation of GTPases, including RAC1, resulting in depolymerization [87–89]. To facilitate the phagosome separation, a concerted effort exists between actin depolymerization and dynamin. Dynamin 2 also directs the trafficking of the full early phagosome [23, 26] downstream.

5.2 Lysosomal degradation

The phagosome and cell corpse are on their way to a well-planned destructive end after being detected and captured by the dying cell [90]. The phagosome is linked to lysosomes, which include proteases, nucleases, and lipases that degrade the phagosome's cargo. Changes to the phagosome, as discussed below, either trigger or inhibit this fusion. In a multi-stage maturation process, the serine/threonine

kinase VPS15 increases the catalytic activity of the early phagosome membrane and the targeting of VPS34 [27, 91]. VPS15 binds to the active RAB5 directly and forms a complex with VPS34 [92]. The role of VPS34 in phagocytosis associated with microtubule-associated protein 1A/1B light chain 3 (LC3) is discussed further (LAP). The transition from early to late phagosomes is characterized by the acquisition of distinct biochemical markers, including small RAB7 GTPase markers, as well as the loss of early RAB5 markers. Late phagosomes are more acidic than early phagosomes due to increased proton pumping into the phagosome lumen mediated by the multimeric protein complex vacuolar ATPase, which translocates H+ across endosomal and phagosomal membranes [36, 47]. RAB7 and its effectors are needed for phagosome maturation, as RAB7 inhibition prevents phagosome-lysosome fusion and results in phagosome acidification failure [32, 91]. RAB7-interacting lysosomal protein (RILP) and oxysterol-binding protein-related protein 1 (ORP1; also known as ORPL1) are found in late endosomes, phagosomes, and lysosomes [32]. By communicating directly with the molecular motor dynein-dynactin, these proteins organize microtubule-dependent vesicular trafficking of RAB7-positive compartments. RILP, dynein, intact microtubules, lysosome-associated membrane protein 1 (LAMP1), and LAMP 2 are involved in the fusion of late phagosomes with the lysosomal compartment [37]. LAMP1 and LAMP2 are highly glycosylated membrane proteins that keep the lysosomal membrane intact and are needed for phagosome-lysosome fusion through interaction with RAB77 [37].

The formation of a Ca2+−dependent SNARE complex, composed of VAMP7 and syntax 7, aids the direct fusion of the mature phagosome with the lysosome [38, 39]. Syntaxine 7 and VAMP7 are both recruited to phagosomes, and phagosome–lysosome fusion is prevented when syntaxine 7 is knocked out [74, 76, 93–98]. The newly developed phagolysosome is distinguished by its high acidity (pH 4.5–5.0) and the presence of active cathepsins after fusion. Acidification is needed for the activation of lysosomal acid hydrolases, which promotes the degradation of the internalized cell body. In conjunction with other effectors such as oxidants, cationic peptides, and lipase, these hydrolytic enzymes mediate the lysosome's potent destructive potential [40, 33].

5.3 Phagosome maturation related modifications

5.3.1 LC3 assisted phagocytosis

The phagosome may be changed to either promote or hinder maturation, affecting the degradation of phagosome material after fusion with lysosomes. During the LAP, one such shift occurs. A subset of proteins necessary for canonical autophagy in LAP, in combination with LAP-specific regulators, allows the LC3 family to be combined with the LAP family. LC3 family proteins facilitate autophagosomes in autophagy, as LC33 depletion also inhibits lysosome fusion [34]. FYCO1, a RAB7 effector protein, interacts with LC3 and PI3P to promote autophagosome-lysosome fusion [35]. Similarly, phagosome LC3 promotes phagosome-lysosome fusion to form a phagolysosome. The rubicon homologue PLEKHM1 interacts with both RAB7 and LC3 and can promote phagosome and endosome trafficking [48, 99]. These processes are most likely shared by autophagia and phagocytosis, both of which are involved in lysosomal targeting and fusion. To date, it has not been thought that the ability to conjugate LC3 with the phagosome directly affects the lysosome's functional potential: impaired maturation and degradation seen in LAP-deficient cells and animals has not been linked to direct lysosomal defects [49, 50].

5.4 Liver X receptors

Other pathways with roles in efferocytosis include the liver X receptors (LXRs) and the PPARs, nuclear receptor families that control genes involved in lipid metabolism and transport [100–103]. By engulfing a dying cell, a phagocyte can effectively double its lipid content, requiring an appropriate transcriptional response which is mediated by these nuclear receptors. LXR deficiency results in their inability to clear the apoptotic thymocytes in macrophages, but does not alter the swallowing of inert beads or other substrates [104]. Similarly, mice that are deficient in LXRs in many tissues accumulate dead cells due to failure of in vivo efferocytosis [104]. Pharmacologic LXR agonists increase efferocytosis and clearance by increasing the expression of receptors, including MERTK, both in cell culture and in vivo136. In addition, during efferocytosis, the existence of LXRs induces the expression of ABCA1 cholesterol transporters, which emit excess cholesterol [105], and genes involved in mitochondrial lipid oxidation, enabling excess lipids to catabolize effectively [105, 106]. Efferocytosis is caused similarly to LXRs by the PPAR family. In response to apoptotic cell engulfment, PPARγ expression occurs and remains elevated until efferocytosis [107] has been resolved. In combination with activation of LXRs, PPARγ activation has been demonstrated to enhance efferocytosis by macrophages [104]. In fact, inhibition of PPARγ by a pharmaceutical antagonist resulted in impaired efferocytosis [107].

5.5 Resolution of phagocytosis

After the phagosome has fused with lysosomes and its cargo has been degraded, a resolution mechanism restores homeostasis within the phagocyte, allowing for further phagocytosis. Since dying cells are not the only phagocytic cargo, the effect of phagocytosis can differ depending on what cargo is internalised. After lysosomal degradation in the form of efferocytosis, some of the components of the cell corpse can be recaptured and recycled for use by the phagocytic cell. Carbohydrates, amino acids, lipids and nucleotides are recycled to replenish cellular stores and can potentially be used as building blocks and sources of energy by the phagocytic cell [108]. The internalisation and degradation of cargo can also influence the activation of signalling pathways; for example, DNA that escapes degradation can activate the DNA sensing cGAS-STING pathway and contribute to the production of type I interferons, as discussed earlier. These events may lead to the loss of the immune tolerance of the apoptotic corpse and can potentially result in autoimmunity [109]. As described earlier, LAP facilitates rapid maturation and generation of phagolysosomes and efficient degradation of cells and promotes the production of anti-inflammatory cytokines, including IL-10, a method that promotes immune silence. On the other hand, LAP disruption decreases phagosome maturation, contributing to the activation of inflammatory signalling pathways and to the production of proinflammatory mediators for IL-1β and [110–113]. The exact mechanism that leads to this switch in cytokine production has not been well known in the absence of LAP. Furthermore, failure in LAP-deficient phagocytes to effectively degrade the cell corpse can result in the leakage of phagosome contents, such as DNA, into the cytosol, which in turn induces the production of type I interferon via STING [114] sensing. STING-dependent interferon expression by tumour-related macrophages in LAP-deficient mice in several cancer models promoted T-cell-mediated anticancer immunity [114]. Following efferocytosis, the phagocyte must restore the functions of cytoskeletal components such as actin and microtubules to ensure that its phagocytic capacity is maximised for additional [115–118]. This restoration of function has not been well studied in contrast with the upstream processes of

phagocytosis. However, such details can be inferred from studies investigating the effectors such as RAB27A in the events leading to phagocytosis. RAB27A negatively affects phagocytosis by prolonging the actin coating of nascent phagosomes, resulting in impaired transition to the stage of phagosome sealing [119]. Therefore, RAB27A and related effectors may be essential for the restoration of actin and other cytoskeletal networks following the completion of phagocytosis. It is evident, however, that cytoskeleton restoration after phagocytosis is an ATP-dependent process and may involve multiple components [120]. An additional function of recovery is the recycling or continued expression of membrane receptors to accept subsequent cargo. Impaired recycling results in reduced membrane expression of receptors such as TREM2 and TLR4, which recognize a variety of amyloids and pathogen products [121, 122] respectively, and can also reduce the membrane expression of dying cell recognizing receptors. This decrease in the available surface receptors can impair the endocytic and phagocytic capacity of the phagocyte cell, which means that these restorative events are necessary to ensure that the phagocyte will continue to recognize and clear phagocyte cargo.

6. Disease and efferocytosis

The fact that efferocytosis has so much redundancy in the detection of dying cells emphasizes its significance in pathophysiology. In reality, many autoimmune and inflammatory disorders are associated with defects in this stage, with inflammation, aggravated tissue damage, and organ dysfunction resulting in uncleared or improperly cleared cell bodies. Defects in other aspects of the efferocytotic machinery, on the other hand, could result in enhanced protection against certain diseases.

6.1 Systemic disease

The most common autoimmune disorder associated with compromised efferocytosis is systemic lupus erythematosus (SLE), a chronic systemic autoimmune disease that affects multiple organ systems such as the lungs, kidneys, skin and central nervous system (CNS). Although uncleared dead cells are rarely detected when healthy tissue is examined under the microscope, dead cells can often be observed in the blood, skin, and lymph nodes of SLE patients, and the seriousness of the disease is closely correlated with defective in vitro efferocytosis and dead cell accumulation in vivo [123, 124]. Patients with SLE also exhibit high levels of circulating autoantigens, such as extracellular DNA, that bind autoantibodies to form immune complexes that accumulate or are deposited in the glomerular and vessel walls of the kidneys [125, 126]. Defects at almost every level of the efferocytosis pathway are involved in the pathogenesis of SLE. Mice with genetic deletion that abrogates find-me signalling pathway components (e.g., development of S1P), eat-me signalling pathway components (e.g. TIM4, MFGE8, S protein, MERTK and C1q) and dead cell treatment components (LXRs, PPARs, ABCA1 and rubicon) all display progressive SLE-like disease.

6.2 Neurodegenerative diseases

Phagocytic cells called ‘microglia’ are present in the CNS. The phenotypically close to these resident phagocytes are the macrophages and clear dead cells and cellular debris in the CNS [127]. However, other CNS cells, such as oligodendrocytes, astrocytes, and neuronal progenitors, can also act as mediators of efferocytosis. Like all organ systems, the CNS needs effective efferocytosis for homeostasis, but

efferocytosis is also essential for the reorganization of the neuronal circuits and for the initiation of regenerative response after injury [128]. Consequently, there are numerous neurodegenerative diseases associated with efferocytosis abnormalities, including multiple sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease [129, 130]. Multiple sclerosis may result from defects that are involved in cell corpse processing at multiple stages of efferocytosis, including pannexin 1find-me signalling defects, MERTK eat-me signalling, and LXR alpha and LXRβ signalling. Decreased expression of the bridging molecule MFGE8 was also observed in a mouse model of Alzheimer's disease and expression of the dominant negative MFGE8 mutant in microglia cells decreased swallowing of apoptotic neurons [131, 132].

6.3 Lung inflammation

In inflammatory diseases, the production of reactive oxygen species (ROS) by neutrophils is increased. ROS can induce RhoA signalling in phagocytes, thereby reducing the clearance of apoptotic cells through inhibition of efferocytosis. A number of lung inflammatory disorders, including chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis, and cystic fibrosis, may lead to impairments or inefficiencies during inflammation in the effective clearance of dying neutrophils [133].

On the other hand, antioxidants such as N-acetylcysteine (NAC) can increase the clearance of apoptotic cells by inhibiting ROS growth, thereby suppressing RhoA activity and increasing the output of TGF-β [134]. The swallowing and clearance of apoptotic leukocytes can also be enhanced by Macrophages [77, 135–139] by anti-inflammatory drugs such as corticosteroids. In particular, protein S binding to apoptotic cells and MerTK (from the TAM family) upregulation on the surface of macrophages can mediate increased clearance of apoptotic debris induced by corticosteroids.

6.4 Atherosclerosis

Lipid oxidation and retention can cause local inflammation in the arterial wall and encourage atherosclerosis progression. Circulating monocytes can aggregate and differentiate into macrophages in atherosclerotic lesions, which can in turn swallow large amounts of lipids and transform into foam cells filled with fat droplets. Macrophage clearance of apoptotic cells occurs primarily in the intima in the early phases of atherosclerosis, and apoptotic cells are cleared quickly and effectively. The condition is different in the case of advanced atherosclerotic lesions (called plaques), where a large number of monocytes/macrophages are recruited into clear foam cell bodies and additional debris. In foam cell bodies, a large number of oxidised lipids are identified which can inhibit the process of efferocytosis by binding to CD14 and can increase the activity of Rho kinase in atherosclerotic [140, 141]. As a result, macrophages do not effectively complete the process of efferocytosis; they thus transform themselves into foam cells and then promote inflammatory responses by secreting pro-inflammatory cytokines, [109, 121, 142–144] and ROS. The clearance of apoptotic cells also decreases and progression towards secondary necrosis increases, thereby sustaining an unfavorable cycle that leads to atherosclerosis progression and associated complications [145–147].

Therefore, macrophages and the efferocytosis system play a very important role in the development of atherosclerosis [135–139]. The accumulation within the plaques of apoptotic cells and the acceleration of atherosclerotic disease are the result of a defect in the separate mediators of efferocytosis [148]. A study showed,

for example, that Gas deficiency contributed to the development of atherosclerosis by reducing the recruitment of lesion phagocytes, while the Mer function defect prevented the clearance of apoptotic cells, which promoted the progression of atherosclerosis. Therefore, a detailed knowledge of the efferocytosis pathways regulated by this cell will open up promising windows for the treatment of atherosclerotic disease because of the critical role of macrophages in atherosclerosis pathogenesis [149].

6.5 Cancer

Although efferocytosis plays a vital role in tissue homeostasis, since clearance of apoptotic cells is followed by immunosuppressive responses, its role in cancer can be detrimental to the host. Indeed, tumour tissue is subject to high-speed cell proliferation and apoptosis, and the process of apoptotic cell clearance, which is associated with anti-inflammatory responses, can encourage tumour escape from immune surveillance and encourage tumour progression. Therefore, some studies have shown that different ligands and receptors involved in efferocytosis may be over-expressed in certain tumour cells and may cause tumorigenesis [40]. Soki et al. have shown that prostate cancer patients' tissue and serum exosomes have a higher degree of MFGE8 expression than controls and that efferocytosis caused by MFGE8 leads to macrophage polarisation towards an M2 phenotype that promotes tumour [33]. Furthermore, in human oral squamous cell carcinoma (SCC) surgical specimens, the immunohistochemical expression of MFGE8 has been associated with some clinical tumour characteristics, including size, stage and invasion patterns [34]. Intensified Mer TK signalling in tumour tissues has been associated with cancer development, metastasis, and resistance to care [35]. Some studies have also been carried out showing that inhibiting the activation of efferocytosis against cancer progression can be successful.

In the co-cultivation of prostatic cancer cells and macrophages, the use of antibodies against MFGE8 substantially decreased the conversion of macrophages to the tumor-promoting M2 phenotype [33]. Tumor production has been minimized in mice by monoclonal antibodies to MFGE8. Consequently, inhibiting efferocytosis and preventing the expression of anti-inflammatory interleukins will improve the immune system's efficacy and anti-tumor effects [99]. However, literature data on the impact of efferocytosis on cancer is troublesome, as there is also evidence that cancer progression may be promoted by a defective efferocytosis. For instance, cancer cells have been shown to express high levels of CD47 on their surface in order to escape from phagocytosis. Therefore, a new approach to cancer treatment, based on inhibiting the expression of CD47 by siRNA or monoclonal antibodies in tumour cells, has emerged to prevent the transduction of don't-eat-me signals into macrophages and increase the phagocytosis of cancer cells [48, 49]. A significant point on this controversial topic is linked to anti-cancer medications. These drugs cause the death of cancer cells by [150–152]. Anti-cancer drug-induced cancer cell apoptosis, accompanied by increased expression of anti-inflammatory cytokines (TGFβ, PGE2, PAF and IL-10), is triggered by efferocytosis in the tumour setting. As a result, this process will lead to the silent anti-tumour response of the immune system [50]. In addition, it has been shown that anthracyclines (e.g., doxorubicin) promote the expression of eat-me signals on the surface of tumour cells, leading to immunogenic cell death [5]. Additionally, as described, during the apoptotic process, apoptotic cells begin to release ATP and UTP within 2 to 4 hours. These nucleoside triphosphates have been demonstrated to act as chemo attractants and to inhibit inflammatory responses in the recruitment of macrophages [11]. The use of chemotherapy drugs to induce apoptosis in tumour cells can increase the

release of nucleoside triphosphates up to 100 times higher, with a significant effect on inflammatory inhibition and suppression of the immune system, although the amounts of nucleotides released during apoptosis are low, so that their effects on immune response suppression are limited. Therefore, a serious reconsideration of anti-cancer drugs is anticipated in the coming years, by fully understanding the function of these agents in apoptotic tumour cell efferocytosis [51, 52] and the related therapeutic consequences.

7. Conclusion

From the point of view of molecular biology and its defects in the development of different diseases, this chapter explores the mechanism of efferocytosis. Just a few examples of the potential effect of disrupted efferocytosis on human diseases are cancer, autoimmune diseases, lung inflammation and atherosclerosis. However, impaired clearance of apoptotic cells, including neurodegeneration, skeletal alterations, cardiac growth, electrical impulse propagation and post-injury recovery, angiogenesis and wound healing, is believed to be involved in many other diseases with unfavorable prognosis and processes. In general, in an inflammation-silenced environment, efferocytosis involves many cells and molecular steps leading to the removal of apoptotic cells. Find-me and eat-me signals are secreted by apoptotic cells for this function, while phagocytic cells often work to clear apoptotic debris with the aid of bridging molecules. Anti-inflammatory interleukins are secreted after the engulfment of apoptotic cells. In fact, cancer progression can be increased by both defective and enhanced efferocytosis. Overall, it is possible to inhibit or activate the receptors associated with this process with accurate knowledge of the molecular mechanisms involved in the efferocytosis process and to target strongly for a new window in the treatment of many human diseases.

8. Future perspectives

Efferocytosis is regulated by a number of factors, including complex membrane lipids and multiple effector proteins. These factors are responsible for functions including dead cell recognition, phagocytosis activation, and, finally, cell corpse degradation. The multi-step process from cell death to cell clearance, as previously mentioned, is delicate, demonstrating the importance of efferocytosis in development, homeostasis, and pathophysiology. The ability of phagocytes to clear cellular debris is required for the homeostatic function of nearly every major physiological system. Efferocytosis dysfunction induces serious disease, while activation of efferocytosis encourages immune silence by processing anti-inflammatory signals, clearing apoptotic cells, and involving the LAP machinery. Since current research indicates that efferocytosis and LAP involvement are beneficial in many environments, inducing an inflammatory response in the treatment of various malignancies by inducing LAP and efferocytosis may be beneficial. The production of therapeutics targeting components of these pathways can increase the efficacy of current cancer therapies such as checkpoint blockade by raising the immunogenicity of apoptotic tumor cells. Since efferocytosis and associated molecular mechanisms are still being studied, new insights into the activation and regulation of the dying cell response are likely to lead to the discovery of new cancer, autoimmunity, neurodegeneration, and beyond treatment paradigms. Efferocytosis is regulated by a number of factors that mediate functions such as dead cell identification and activation of the multi-step process from cell death to cell clearance, demonstrating

the importance of efferocytosis in growth, homeostasis, and pathophysiology. The ability of phagocytes to clear cellular debris is required, at least in part, for the homeostatic function of almost every major physiological system. Efferocytosis dysfunction causes severe disease, while activation of efferocytosis promotes immune silence through anti-inflammatory signal processing in the context of clearing apoptotic cells and the involvement of the LAP machinery. Since current research suggests that efferocytosis and LAP involvement are beneficial in a variety of settings, inducing an inflammatory response by inducing LAP and efferocytosis can be beneficial in the treatment of various cancers. Established cancer therapies, such as checkpoint blockade, may be improved by developing therapeutics that target components of these pathways, such as enhancing the immunogenicity of apoptotic tumor cells.

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