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PHAGOCYTOSIS OF APOPTOTIC CELLS IN MAMMALS, *CAENORHABDITIS ELEGANS* AND *DROSOPHILA MELANOGASTER*: MOLECULAR MECHANISMS AND PHYSIOLOGICAL CONSEQUENCES

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1. ABSTRACT

Phagocytosis is the necessary corollary of apoptosis. It leads to the clearance of apoptotic cells by phagocytes, which can be 'professional' or 'amateur'. I review the known molecular aspects of phagocytosis of apoptotic corpses in mammals, *Caenorhabditis elegans* and *Drosophila melanogaster* from the point of view of the phagocyte and the apoptotic corpse. I highlight recent advances made in the field and discuss the physiological outcomes and consequences of this process. Indeed, phagocytosis of apoptotic cells is important in shaping or remodeling tissues to maintain their integrity and specialized functions during development and wound healing. It also contributes to the development of inflammation and/or its resolution after an injury or infection. This perhaps explains why the molecular mechanisms of phagocytosis of apoptotic cells are redundant and complex in mammals and suggests why they appear to have been mostly conserved through evolution. *Caenorhabditis elegans* has already proven to be useful in genetically dissecting the molecular mechanisms underlying phagocytosis of apoptotic corpses by 'amateur' neighboring cells. *Drosophila melanogaster* will become the model of choice in genetically dissecting the molecular mechanisms underlying phagocytosis of apoptotic cells by 'professional' phagocytes such as macrophages.

2. INTRODUCTION

The rapid recognition and ingestion of dying cells, so-called apoptotic corpses or bodies occurs by phagocytosis. Apoptosis is the process by which cells are programmed to die in a timely and orderly manner (1-3). A significant overproduction of cells occurs at all stages of development in all organisms, and thus apoptosis is critical in the regulation and maintenance of homeostasis and tissue integrity (1-3). Apoptosis is also important for the swift removal of cells with damaged DNA. Studies of the initiation and execution of the apoptotic program have attracted considerable interest over the past two decades. The use of animal models such as *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster*, with the advantage of powerful genetics, has greatly contributed to advances made in this field (4-6).

The importance of phagocytosis of apoptotic cells has often been underestimated. Most cells that undergo apoptosis are ingested *in situ* by either 'professional' or 'amateur' (non-professional) phagocytes. The combined efforts of many laboratories and the use of model organisms, in particular *C. elegans*, have led to the revelation of some of the molecular mechanisms by which this occurs (7-9). But why are such efforts made?

Phagocytosis of apoptotic corpses has often been considered as a mere housekeeping function, yet it plays a critical role in shaping organs during development, as well as in tissue remodeling during wound healing. There is now growing evidence that the orderly removal of apoptotic corpses is more crucial than originally thought. Apoptotic cells are generally rapidly removed, prior to the loss of their plasma membrane integrity, thus preventing

damage caused by accidental leakage of their potentially cytotoxic or antigenic content (1, 10). However, several studies in mammals showed that macrophages phagocytosing apoptotic cells can also trigger further programmed cell death of bystander cells (11-13). Further, recent studies in *C. elegans* have demonstrated that the uptake itself may accelerate apoptosis of cells not yet fully committed to die (14, 15).

Phagocytosis of apoptotic cells is usually accompanied by anti-inflammatory signals, which are critical in the resolution of inflammation and the regulation of immune responses (16). In some circumstances, however, clearance of apoptotic cells may also lead to the activation of macrophages and trigger the production of pro-inflammatory cytokines (17, 18). This discrepancy is not yet fully understood. Studies suggest that failure to dispose of apoptotic cells can lead to neurodegeneration, and that it may also contribute to autoimmune diseases (19-23). Interestingly, it was also recently shown that parasites can enter professional phagocytes by routes similar to those taken by apoptotic corpses, and thus subvert the ability of the phagocytes to participate in their killing, as well as that of infected phagocytes, providing the parasites with a favorable environment in which to grow (24, 25).

Finally, more recent findings suggest that the uptake of apoptotic leukemia cells by dendritic cells (DCs) may have a protective role against leukemia *in vivo* (26). Similar findings using DCs that have engulfed apoptotic/necrotic melanoma cells further the hope of new strategies in cancer vaccine development (27). In contrast, the uptake of apoptotic cells expressing an activated oncogene could lead to its horizontal transfer into the phagocytes, which may in turn develop a tumorigenic phenotype (28). The complexity and importance of phagocytosis of apoptotic cells therefore deserves our attention.

This review is an attempt to summarize the current knowledge of the molecular mechanisms of apoptotic cell clearance while highlighting recent advances made in the field, and to discuss the many outcomes and unknowns of this important biological process.

3. MOLECULAR MECHANISMS OF PHAGOCYTOSIS OF APOPTOTIC CELLS IN MAMMALS: A GREAT COMPLEXITY

3.1. Professional phagocytes and receptors

Both ‘amateur’ and ‘professional’ phagocytes mediate phagocytosis of apoptotic cells. ‘Amateur’ phagocyte generally refers to ‘on scene’ cells that are capable of recognizing and engulfing an apoptotic cell. However, the term of ‘amateur’ can also be employed for cells that are poorly phagocytic, whether or not they have the ability to migrate to the site of apoptosis. In contrast, professional phagocytes are generally mobile cells that can infiltrate various tissues and have a high phagocytic activity.

Macrophages or neutrophils are the professional phagocytes. A large number of surface receptors have been identified on these cells that participate in phagocytosis of apoptotic cells. On macrophages, these include (i) the alpha-v/beta-3 integrin, or vitronectin receptor (29), (ii) the scavenger receptor of class B, CD36 (30-32), (iii) the scavenger receptor of class A, SR-A (33), (iv) the glycoprotein macrosialin/CD68 (34), (v) the ATP-binding cassette transporter 1, ABC1 (35, 36), (vi) the cluster of differentiation 14 (CD14)(37), (vii) the phosphatidylserine-receptor (PS-receptor)(38), (viii) the surfactant protein A (SP-A), a collectin (39), (ix) the MER receptor tyrosine kinase (22), as well as (x) a recently identified protein complex that involves calreticulin (CRT), (also called collagenous tail binding C1q receptor (cC1qR)), and the endocytic receptor CD91, or low density lipoprotein (LDL) receptor-related protein (LRP), (also known as the alpha-2 macroglobulin (alpha-2 m) receptor)(40). Furthermore, IgG-opsonised apoptotic cells can be recognized and engulfed via both the complement receptor CR3 and the Fc-gamma receptor pathways (21, 41-43)(Figure 1).

Most of these receptors were characterized by inhibition studies using apoptotic ligands or monoclonal antibodies. Interestingly, several of these proteins bind a wide variety of ligands. For instance, ABC-transporters belong to the largest family of transporters, and translocate a wide range of sugars, amino-acids, metal ions, peptides, proteins, as well as a large number of hydrophobic compounds and metabolites across membranes (44). Mutations in ABC-transporters thus affect multiple cell functions and 14 out of the 48 known human genes have been associated with genetic disorders such as cystic fibrosis, neurological diseases, retinal degeneration, cholesterol and bile transport defects, anemia, and drug response (44). ABC1 is unique in that it is found both on the apoptotic cells as well as on the phagocyte (35, 45). ABC1 was also recently shown to participate in the redistribution of phosphatidylserine (PS) on both the apoptotic cell and the phagocyte, although it is not yet known how ABC1 promotes this important lipid redistribution across the membrane (46). Flipping of PS to the outer leaflet of the plasma membrane is an apoptotic cell surface change found on most if not all apoptotic cells throughout the animal kingdom (47). A PS-receptor was recently characterized that participate in phagocytosis of apoptotic cells (38). Scavenger receptors (SRs), a large family of receptors defined by their binding affinity for a wide range of polyanionic ligands, which participate in their endocytosis, also bind PS (34, 48-50).

Several receptors for phagocytosis of apoptotic cells, such as SRs, also play a role in phagocytosis of bacteria and participate in innate immune responses (50). CD14, a leucine-rich glycoprotein, is also one such receptor and was first characterized as a lipopolysaccharide (LPS) receptor that participates in bacterial clearance (51). However, lipid-derived moieties are not the only bacterial and apoptotic ligands. Collectins are calcium-dependent lectins that target carbohydrate structures on pathogens, resulting in the agglutination and

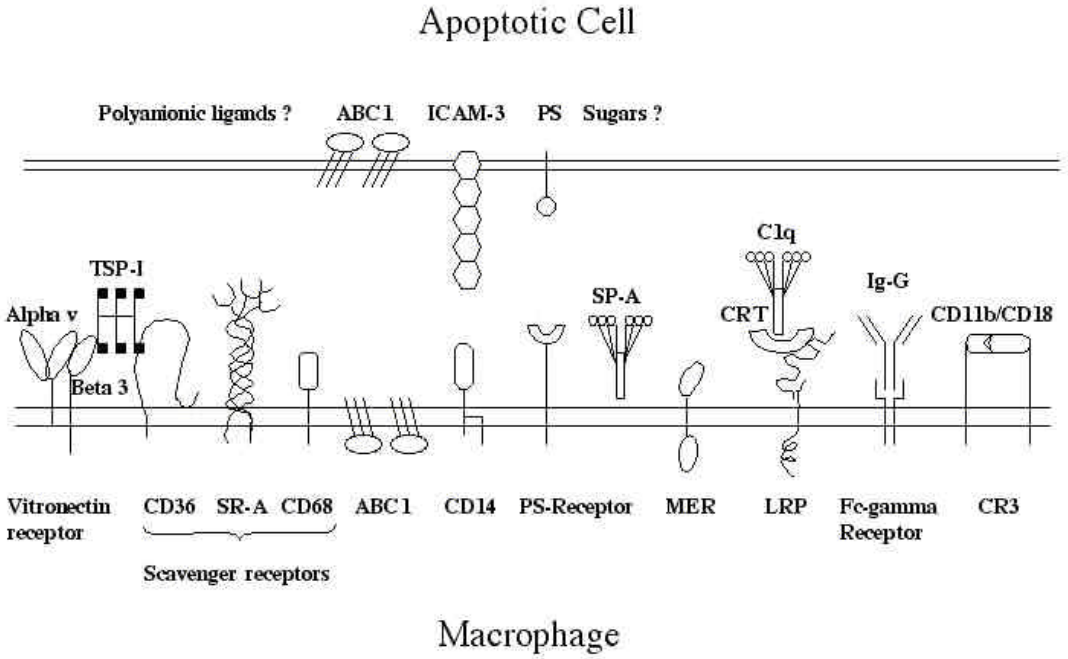

Figure 1. Schematic representation of the mammalian receptors involved in phagocytosis of apoptotic cells by professional phagocytes, the macrophages. The vitronectin receptor is composed of the alpha-v/beta-3 integrins, and interacts with the class B scavenger receptor, CD36 via a bridge of TSP-1, a protein of the extracellular matrix. Other scavenger receptors, such as the scavenger receptor class A, SR-A and CD68 also participate in the clearance of apoptotic cells. The ABC1 transporter is found on both the macrophage and the apoptotic cells. CD14, a GPI-anchored receptor, which binds LPS, also recognizes apoptotic cells via binding of the adhesion molecule ICAM-3, a highly glycosylated Ig-superfamily member. The PS-receptor, a predicted type II transmembrane protein, binds PS, a phospholipid flipped on the outer surface of apoptotic cell membranes. The collectin SP-A and the collectin-related complement protein C1q both participate in the recognition of apoptotic cells, possibly via binding to altered sugars at the surface of apoptotic cells. C1q binds to CRT, a protein of the plasma endoreticulum that is targeted to the surface membrane of the macrophage via its binding to the LDL receptor-related protein, LRP, itself composed of an alpha chain and a membrane spanning beta chain. MER is a tyrosine kinase also found on RPE cells involved in the clearance of ROS photoreceptors, which are shed daily and in a large number in the retina. Both the Fc-gamma receptor and CR3 can also participate in the clearance of apoptotic cells via their opsonization with Ig-G antibodies or complement proteins.

enhanced clearance of microorganisms (52). These trimeric proteins may assemble into larger oligomers. Each polypeptide chain consists of a short amino-terminus, a collagen like region, an alpha-helical coiled-coil, and a carbohydrate recognition domain (CRD). The CRD confers the binding specificity onto lectins, and the CRDs found on lectins with similar binding affinity are generally highly conserved in amino-acid sequence. Inhibition studies have suggested that many lectins with different binding affinities may participate in clearance of apoptotic cells (39, 40, 53-55). Although most are uncharacterized, one collectin, SP-A, was recently shown to participate in the clearance of apoptotic cells by alveolar macrophages (39).

A number of phagocyte receptors cooperate in binding and triggering the uptake of apoptotic cells. CD36 cooperates with the vitronectin receptor alpha-v/beta-3 (31). Other data suggest that CD36 may also cooperate with the PS-receptor (56). Most recently, yet another combination of proteins was unraveled that triggers recognition and uptake of apoptotic cells. An antibody against cC1qR, one proposed receptor for C1q and collectins, effectively inhibited binding of C1q-coated apoptotic cells (40). cC1qR has an almost complete identical amino acid sequence to CRT (57). Although originally identified as an endoplasmic reticulum protein, CRT was recently found on the surface of macrophages (40). cC1qR/CRT does not have a transmembrane domain and thus may require a partner to trigger its localization to the membrane. Interestingly, cC1qR/CRT can bind to LRP, a cell surface receptor, and an anti-LRP antibody also blocked phagocytosis of apoptotic Jurkat cells by macrophages *in vitro* (40, 58). Both cC1qR and LRP co-localized on the cell surface of macrophages (40). C1q and MBL, a collectin, which also inhibited phagocytosis of apoptotic cells, competed in binding to the same receptor on macrophages (40). Using a receptor modulation experiment, Ogden and colleagues showed that C1q, cC1qR/CRT and LRP cooperated to trigger phagocytosis of apoptotic cells by macrophages, although a direct physical interaction between all the partners remains to be formally proven (40). Interestingly, this uptake occurs via a process reminiscent of macropinocytosis, which involves the formation of large macropinocytic vacuoles accompanied with concurrent ingestion of extracellular fluid (40).

Finally, the MER receptor tyrosine kinase was identified by genetic analysis of a mutation that affected phagocytosis of apoptotic cells by amateur phagocytes and will therefore be discussed further below (59). MER was subsequently found on macrophages where it sustains a similar function (22).

3.2. Amateur phagocytes

A large number of 'amateur' phagocytes have been identified in mammals that include some epithelial cells, fibroblasts and endothelial cells, Sertoli cells, microglial cells, renal mesangial cells, as well as immature DCs (1, 10, 60). Many of these cells, in particular epithelial cells, participate in the shaping or remodeling of organs or tissues, and contribute to the maintenance of their integrity and specialized functions. For instance, cells of the retinal-pigmented epithelium (RPE) clear the enormous number of apoptotic rod outer segments (ROS) that are shed daily, and play a key role in maintaining vision (61). In the Royal College of Surgeons (RCS) rat, RPE cells fail to recognize and engulf shed ROS (62, 63). This defect leads to subsequent apoptosis of cone and rod photoreceptors and results in degeneration of the retina, which ultimately causes blindness (61). Similarly, olfactory epithelial cells may play a role in maintaining olfaction by efficiently clearing apoptotic neurons, which are extensively renewed in the adult olfactory organ (64). Finally, a massive loss of cells by apoptosis occurs during involution of the mammary gland where epithelial cells engulf their dying neighbors (65). This process may be critical for the normal remodeling of the gland in preparation for the next wave of lactation. Macrophages were also observed in these tissues and may participate in the removal of apoptotic cells.

However, apoptosis often occurs in tissues where macrophages do not infiltrate, such as the brain and testis, where the clearance of apoptotic cells is fully accounted for by 'amateur' yet efficient phagocytes. In the testis, the somatic Sertoli cells clear dying spermatogenic cells in the seminiferous tubules (66). The meaning of this apoptotic clearance is not yet well understood, but Sertoli cells play multiple roles in nurturing and supporting germ cells survival (67, 68). In the brain, microglial cells are the primary immune cells of the central nervous system (CNS) where they engulf numerous apoptotic neurons, thereby preventing neurodegeneration and cerebrovascular strokes. Phagocytosis of apoptotic inflammatory cells by microglia may also be an important mechanism for the resolution of inflammatory attacks of the CNS.

Other 'amateur' phagocytes serve as a defense system for a particular tissue or play a critical protective role in the regulation of immune responses after an injury or infection. They can contribute to the development of an inflammation and/or to its resolution. For instance, renal mesanglial cells clear apoptotic neutrophils. A failure of this clearing process results in glomerulonephritis, an inflammation of the kidney that can be temporary and reversible or may be progressive, resulting in destruction of the glomeruli and subsequent chronic or permanent renal failure (19, 69). In the thymus, epithelial nurse cells clear apoptotic thymocytes and participate in the selection of specific lymphocyte populations (70). Fibroblasts phagocytose apoptotic neutrophils, and can also clear a large number of red blood cells after a trauma or during internal bleeding (53, 71, 72). In the liver, hepatocytes, Kupffer cells and endothelial cells can all engulf apoptotic lymphocytes and senescent erythrocytes (73, 74). Sinusoidal cells in particular were shown to be able to phagocytose apoptotic lymphocytes from the circulation, which allows them to participate in the development of a tight and specific response, or the resolution of such a response (75).

Finally and most interestingly, immature DCs can also participate in the clearance of apoptotic cells (76-78). DCs are mobile cells with important immune functions. They are professional antigen-presenting cells (APCs) that participate in the activation of both CD4 and CD8-positive T cells. Although they are competent to phagocytose apoptotic cells, immature DCs are much less efficient phagocytes than macrophages or neutrophils and perform better in their absence. Thus, they are considered as 'amateur' phagocytes. Furthermore, and in contrast to other phagocytes, DCs do not silently destroy the digested apoptotic cells but process them for antigen presentation onto major histocompatibility complexes (MHC) of class I and II (77, 78). Thus, in a normal state, the presentation of apoptotic cell-derived antigens by DCs suggests that they may play a role in cross presentation and tolerance, thereby causing allograft rejections. In contrast, during an inflammation, DCs phagocytose microorganisms and necrotic cells and most likely play key roles in mediating strong T-cell responses.

It is now well accepted that most if not all cell types can become phagocytic when given with the opportunity, and the list of 'amateur 'phagocytes is ever growing. Consistent with this idea is the observation that phagocytosis of apoptotic cells in the interdigits of the footplate of a PU.1 knock-out mouse, which is devoid of macrophages, can still occur and is performed by neighboring mesenchymal cells (79).

3.3. Receptors on amateur phagocytes

Strikingly, most 'amateur' phagocytes use similar phagocytic receptors to those found on macrophages, some of which were indeed first characterized while studying 'amateur' phagocytes. LOX-1, a lectin-like oxidized LDL-receptor, is one such example and is found on vascular endothelial cells where it participates in the clearance of apoptotic neutrophils (54). Interestingly, this receptor is also expressed on macrophages, but it is not yet known whether it is needed for clearance of apoptotic bodies by those cells. RPE cells utilize the alpha-v/beta-5 integrin in binding of the shed photoreceptors and require CD36 for internalization (80-83). Cultured RPE cells could also trigger the serum-stimulated uptake of ROS via a mechanism involving vitronectin (VN)(84). Interestingly, the mutation of the RCS rat, whose RPE cells fail to remove apoptotic shed photoreceptors, was recently identified as a small deletion

that disrupts the gene encoding a receptor tyrosine kinase of the Axl/Mer/Tyro3 family, *Mertk* (59). Mutations in the human orthologue of *Mertk* were also found in patients with retinitis pigmentosa where a similar defect in phagocytosis of shed photoreceptors was observed (85, 86). The MER receptor tyrosine kinase receptor was therefore proposed to play a role in phagocytosis of apoptotic ROS by RPE cells (59). A subretinal injection of a MER-expressing virus in the RCS rat allowed for a partial and transient but significant correction of the retinal dystrophy phenotype associated with this mutation, further demonstrating an *in vivo* role for MER in phagocytosis of apoptotic photoreceptors by RPE cells (87).

Soon after its characterization, MER was also found on monocytes and macrophages (22). Macrophages from a functional knockout mouse (*mer kd*), carrying a MER protein with a truncated cytoplasmic tail, failed to efficiently clear apoptotic thymocytes *in vivo* (22). These cells showed a defect in engulfment, rather than in binding of apoptotic cells, which suggests that the cytoplasmic tail of MER is required to trigger the uptake of apoptotic cells but not for their proper recognition by macrophages. Therefore not all receptors are able to trigger the uptake of apoptotic cells but rather serve as recognition molecules. A 'tethering and tickling' model was proposed whereby some receptors anchor the apoptotic cells while others are subsequently recruited or activated to signal and trigger the cytoskeleton changes required for the extension of the membrane around the particle during formation of the phagosome (88). This model would partly explain the diversity of receptors found on phagocytes and their ability to interact or cooperate in the binding and uptake of apoptotic cells.

It is becoming apparent that different phagocytes can use various combinations of receptors to mediate binding and uptake of apoptotic bodies. For instance, Sertoli cells use a CD36-related scavenger receptor, the scavenger receptor of class B type 1 (SR-B1) (80, 89). However, a lysosomal ABC-transporter related receptor, ABCB9, was also recently characterized in human and rat that is highly expressed on these cells and may also be involved in phagocytosis of apoptotic spermatogenic cells (90). In the thymus, nursing epithelial cells also use the CD36-related scavenger receptor, SR-B1, in clearance of apoptotic thymocytes (91). DCs were found to use both CD36 and the alpha-v/beta-5 integrin (76). Mesanglial cells utilize yet another integrin, the vitronectin receptor alpha-v/beta-3 (69). Fibroblasts engulf apoptotic neutrophils via a mechanism that also requires the alpha-v/beta-3 integrin and a mannose/fucose lectin (53). Microglial cells-mediated phagocytosis of apoptotic neurons is inhibited by N-acetylglucosamine or galactose, suggesting the involvement of asialoglycoprotein-like lectins; by the RGDS peptide, arguing in favor of a role for the vitronectin receptor; and finally by PS or O-phospho-L serine enriched vesicles, implicating a role for a PS-receptor (55). In the liver, engulfing cells essentially use lectin-like receptors, which recognize galactose and mannose residues. Indeed, it has been shown that up-regulating the cell surface expression of mannose receptors on liver endothelial cells enhances their ability to clear apoptotic lymphocytes (55).

Why phagocytes employ various combinations of receptors is unclear. One explanation is that most of these cells are part of a specific tissue or organ and thus participate in specialized functions, needing different specific molecules on each of these cells. Phagocytes may use different receptors depending on the nature of the apoptotic cell or its apoptotic stage, or of their own state of activation. They may also simply have redundant recognition and uptake mechanisms, which could become useful in the event of overwhelming apoptosis or be used as back-up systems in the event of defective mechanisms of uptake (92, 93). This diversity of receptors, in particular of those that are also employed in innate immune defenses, might also have been driven by pathogen variation. Phagocytic receptors for pathogens would have then adapted to be exploited by the host and participate in the recognition and engulfment of various apoptotic cell types.

3.4. Apoptotic cells surface ligands or 'eat me signals', opsonins and bridging molecules

Despite their characterization, the molecular mechanisms by which these numerous phagocytes and their receptors participate in the recognition/binding and engulfment/uptake of apoptotic cells is still poorly understood. This is partly due to the incomplete characterization of the cell surface changes on dying cells that these receptors recognize.

Cells dying by apoptosis undergo major morphological changes, such as cell shrinkage and rounding accompanied by cytoplasm and nuclear condensation, internucleosomal DNA fragmentation, as well as membrane blebbing (94-96). The maintenance of membrane integrity is an important hallmark of apoptosis, yet profound cell surface changes occur that are important for the recognition and engulfment of apoptotic cells by phagocytes (94). These include: (i) alterations in carbohydrates (53, 73, 97), (ii) the important and almost universal loss of membrane phospholipid asymmetry resulting in increased exposure of the anionic phospholipid, PS (98, 99), as well as (iii) changes in the adhesion molecule ICAM-3 (also known as CD50) (100). The nature of these molecular alterations accounts for the nature of the receptors involved in the clearance of apoptotic corpses. Thus, lectins participate in the recognition of carbohydrate pattern alterations. CD14 binds to ICAM-3 (100, 101). PS-receptor and scavenger receptors bind to PS (38, 48, 49). The ability of several receptors to bind the same ligand adds yet another level of complexity and security in the recognition and uptake of apoptotic cells. On one hand, this could result in two receptors competing for the same ligand and could affect the efficiency by which a phagocyte recognizes its target. On the other hand, if a large number of ligand molecules are present at the surface of apoptotic cells, their recognition by several receptors on the phagocyte may simply strengthen binding and favor rapid uptake.

Not all phagocytic receptors act by directly binding to a unique and specific ligand at the surface of apoptotic cells. Molecules bridging two receptors on the phagocyte or opsonins on the apoptotic cells are sometimes required. These molecules may also strengthen the interaction between an apoptotic corpse and a phagocyte. For instance, thrombospondin-1 (TSP-1), a multimeric glycoprotein that functions as an adhesion molecule and a component of the extracellular matrix, was shown to act as a molecular bridge between the vitronectin receptor alpha-v/beta-3 and CD36 in phagocytosis of apoptotic cells by macrophages (31, 102). TSP-1 and alpha-v/beta-3 are also required for phagocytosis of apoptotic cells by mesanglial cells, although this interaction occurs in a CD36-independent manner (69). TSP-1 binds to the apoptotic corpse, although the surface molecule to which it binds is not yet known.

Opsonising molecules include the anti-apoptotic GAS6 and the beta2 glycoprotein I (beta2GPI) (19, 103). GAS6 is a growth arrest specific gene implicated in the negative regulation of blood coagulation cascade, and participates in cell proliferation and survival, as well as cell adhesion and chemotaxis (104-108). Most recently, GAS6 was shown to enhance platelet aggregation and secretion and its blockage or deletion allowed for a protection against thrombosis (109). Interestingly, GAS6 is a ligand for receptor tyrosine kinases of the Axl, Sky and Mer family (110-113). GAS6 enhances the uptake of PS, as well as apoptotic cells by macrophages (114). This enhancement requires its interaction with the surface of the macrophage, presumably through a receptor tyrosine kinase. While GAS6 could bind PS, its physiological ligand on apoptotic cells remains uncertain. Beta2GPI, a serum glycoprotein, also binds PS, and macrophage uptake of apoptotic cells was enhanced in its presence (115, 116). This uptake was further increased by addition of beta2GPI antibodies (115, 117). Why this occurs is not well understood.

Two additional molecules, the first component of the complement cascade, C1q, and a member of the collectin family of molecules, the mannose-binding lectin (MBL) were also recently shown to act as opsonins, promoting phagocytosis of apoptotic cells. Humans with C1q deficiency show an increased risk for bacterial infections and autoimmune diseases such as systemic lupus erythrematosus (SLE) and glomerulonephritis, both of which are accompanied by elevated levels of free apoptotic cells and auto-antibodies production that could be the result of a defect in clearance of apoptotic cells (19, 21). Similarly, C1q knockout mice developed autoantibodies and glomerulonephritis, and showed a defect in clearance of apoptotic cells *in vivo* (19, 118). An MBL deficiency in humans is associated with increased susceptibility to infection and diseases such as chronic hepatitis B viral infection, cystic fibrosis, as well as SLE (119-121). These data suggested a potential role for both C1q and MBL in phagocytosis of apoptotic cells. C1q was shown to bind many apoptotic cell types, including apoptotic vascular endothelial cells and keratinocytes, as well as both non-apoptotic and apoptotic Jurkat cells *in vitro* (40, 122, 123). Interestingly, C1q binding was observed on the entire surface of non-apoptotic cells while it was found to concentrate in the blebbing areas of apoptotic cells, which might be required for its interaction with the phagocyte receptor (40). A possible explanation for this re-localization of C1q is that it may be necessary to trigger recognition of apoptotic cells at a relatively early stage of apoptosis when cells start blebbing. In contrast, MBL bound to apoptotic cells exclusively, and may be used to recognize cells at a later stage of apoptosis when dying cells have fragmented into apoptotic corpses. As expected, both mannosidase-treatment and the addition of mannose inhibited the binding of MBL to apoptotic cells, and decreased the efficiency of their uptake by human monocyte-derived macrophages (40). Antibodies against C1q and MBL at least partially inhibited phagocytosis of apoptotic Jurkat cells *in vitro* (40). C1q and MBL thus seem to act as opsonins to enhance the uptake of apoptotic Jurkat cells by human monocyte-derived macrophages (40). These observations led to the identification of cC1qR and LRP as a new complex of proteins that cooperate in the binding and uptake of C1q-opsonised apoptotic cells (40).

4. PHAGOCYTOSIS OF APOPTOTIC CELLS AND ANIMAL MODELS: A GENETIC DISSECTION

4.1. *C. elegans* and the clearance of apoptotic cells by 'amateur' neighboring cells

The redundancy and complexity in the mechanisms of recognition and uptake of apoptotic cells by phagocytes in mammals has made their molecular analysis difficult and many laboratories have turned to the power of genetics in organisms such as *C. elegans*. Studies using this animal model have already significantly contributed to advances made in the field, in particular with regard to the identification of intracellular components of the phagocytic machinery that signal cytoskeleton rearrangement during engulfment.

The lineage of all cells in *C. elegans* has been thoroughly defined (124, 125). 131 somatic cells and over 300 germ cells undergo apoptosis and are rapidly cleared by their neighboring cells. Failure of cells to recognize and engulf their dying neighbors results in persistence of apoptotic corpses (126). These are easily distinguished because of their characteristic round shape, accompanied by cell shrinkage and dilated nuclei, and appear as refractile disks under Nomarski optics. This has allowed for an easy screening of mutations that affect the engulfment of apoptotic cells (126). At least 6 mutations were first identified that led to the characterization of genes that are required for the engulfment of apoptotic cells. All belong to the *ced* (*C. elegans* death) family of genes and have been conserved throughout evolution with homologues in *Drosophila* and mammals. Genetic analysis identified two complementation groups organizing these mutations in at least two pathways: the CED-1, -6, and -7 pathway, and the CED-2, -5, and -10 pathway (126). Mutants in the latter pathway also showed a severe defect in migration of the distal tip cells (DTCs) in the gonads (127). Most recently, a seventh mutant, *ced-*

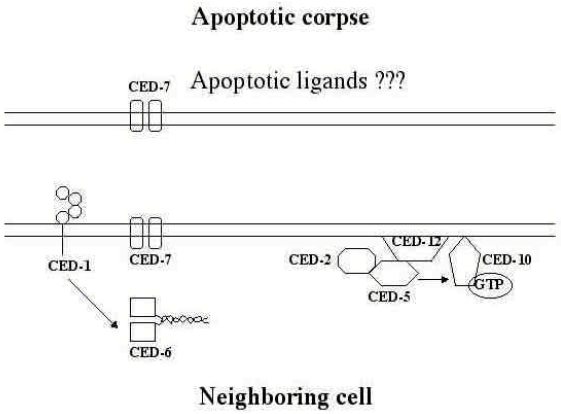

Figure 2. Schematic representation of the two pathways involved in clearance of apoptotic cells by their neighbors in *C.elegans*. CED-1 is a scavenger receptor that shares sequence homologies with the mammalian scavenger receptor SREC, as well as with the LDL-related receptor protein, LRP, recently proposed to be the functional mammalian orthologue of CED-1. CED-1 clusters at the site of binding to the apoptotic corpse and participates in its clearance. CED-6 is an adapter protein that acts downstream of CED-1 and CED-7. Its mammalian homologue GULP was recently shown to interact with both CED-1 and LRP. CED-7 is the ABC1 homologue and acts in the same pathway as CED-1 and CED-6. As for its mammalian counterpart, CED-7 is found on both the apoptotic cell and the phagocyte and is believed to participate in flipping PS at the surface of the corpse. CED-2, CED-5 and CED-10 are homologous to mammalian CrkII, DOCK180 and RAC respectively. CED-2 is an adapter protein that contains both SH2 and SH3 domains. CED-5 has an SH3 domain and interacts with CED-2 and CED-12. CED-12 encodes a protein with a PH domain and SH3 binding motif, and interacts with the carboxy-terminus of CED-5. CED-12 has a mammalian homologue ELMO, which also interact with DOCK180. CED-10 is a small GTPase and acts downstream of CED-2, 5 and 12. The CED-2, 5, 10 and 12 pathway triggers the necessary cytoskeletal changes that trigger cell shape and engulfment by the phagocyte. These proteins are also required for the proper migration of distal tip cells in *C.elegans.*

12, was characterized based on a similar migration defect, and was shown to have a severely reduced engulfment activity, which argued in favor of CED-12 being part of the CED-2, -5 and -10 pathway (128-130) (Figure 2).

All seven genes have been fully characterized. *ced-1* encodes a scavenger-receptor that acts as a receptor for apoptotic cells and shares sequence similarities with human SREC found on endothelial cells (131). CED-1 is required on the engulfing cell and clusters at the site of membrane binding to the apoptotic corpse (131). While CED-1 is homologous to SREC, the mammalian LRP receptor also shares homologies with CED-1 and was recently proposed to be its functional orthologue (132). *Ced-6* encodes an adapter protein with a phosphotyrosine binding (PTB) site, a leucine-zipper motif for homodimerization, and a potential proline-rich motif, and acts downstream of CED-1 and CED-7 (131, 133). Its human homologue GULP was recently shown to interact both with CED-1 and LRP and to participate in phagocytosis of apoptotic cells (132, 134). The *ced-7* gene product is similar to the ABC-1 transporter and is required for CED-1 clustering (129, 131). As for ABC1, CED-7 is also found on the apoptotic cell and believed to act by exposing a phospholipid ligand on the cell surface of the dying cell (131). CED-2 is similar to CRKII, a signaling molecule containing SH2 and SH3 domains (127). CED-5 belongs to the CDM family of proteins, which include CED-5, its mammalian homologue DOCK180 and the *Drosophila* protein myoblast city (MBC), and interacts with CRKII (135-137). CED-5 was proposed to function in the engulfing cell and to trigger the extension of its membrane around the dying cell (136). CED-2 and CED-5 were shown to physically interact *in vitro* (127). *Ced-10* encodes a small GTPase of the Rho/Rac/cdc42 family that is most similar to Rac (127). CRKII, DOCK180 and Rac were all shown to be involved in triggering cytoskeletal changes that lead to the engulfment of apoptotic cells in mammalian systems (138, 139).

Ced-12, the newly identified gene involved in engulfment of apoptotic cells, encodes a protein with a pleckstrin homology (PH) domain and SH3 binding motif (128-130). PH domains are found in many proteins that act in signal transduction pathways or in cytoskeleton reorganization. A *ced-12* mutation strongly exacerbates the engulfment defect seen in loss-of function mutation of *ced-1, 6* and *7*, but does not affect that of a *ced-5* mutant (128-130). Moreover, CED-10 overexpression could bypass the requirement for CED-12, arguing that CED-10 acts downstream of CED-12 in a same pathway (128-130). CED-12 functions in engulfing cells, and was further shown to physically interact with the carboxy-terminal sequence of CED-5 (128). ELMO1, the mammalian homologue of CED-12 also interacted with CED-5, as did CED-12 with DOCK180, the mammalian homologue of CED-5 (129, 130). Consistently, ELMO1 and DOCK180 also physically interacted (130). Using a yeast three-hybrid system, Wu and colleagues further demonstrated that CED-5 acts as a bridge to link CED-2 and CED-12 (129). Whether this complex forms *in vivo* or what might trigger these proteins to interact with each other is not known. Several lines of evidence imply the involvement of Rac and its homologue CED-10 in the signaling pathway that regulates the cytoskeleton dynamics during engulfment in mammals and *C. elegans*, respectively (127, 138, 139). Surprisingly, Zhou and colleagues showed that overexpression of CED-12 in Swiss 3T3 cells could also regulate cytoskeletal rearrangements via yet another small GTPase, Rho (128). While activation of Rac induces polymerization of actin leading to ruffles and filopodia formation, Rho induces actin-assembly into bundles and stress fibers (140). Thus CED-12 might act through different small GTPases to reorganize the cytoskeleton in various functions. What would trigger CED-12 to interact with a particular small GTPase in a specific situation is not understood.

4.2. *Drosophila melanogaster* and the clearance of apoptotic cells by 'professional' phagocytes, the macrophages

Drosophila melanogaster is also often used to genetically dissect the molecular mechanisms of biological processes of interest. While in *C.elegans*, apoptotic corpses are cleared by amateur phagocytes, the neighboring cells, several cell types, both of the amateur and professional classes, have been reported to participate in the clearance of apoptotic cells in *Drosophila* (141) (142-144). Thus, in this respect, *Drosophila* is more similar to mammals and recently became a model organism of choice to study the molecular mechanisms of apoptotic cell clearance by professional phagocytes (145, 146).

In *Drosophila*, epithelial and glial cells are the amateur phagocytes, and macrophages, also called plasmatocytes, are the professional phagocytes (141-144). *Drosophila* macrophages have similar functions to their mammalian counterparts: they participate in phagocytosis of apoptotic cells during development, as well as of microorganisms in innate immune responses, and have scavenger activities (141, 146-149). They are also the major producers of extracellular matrix proteins and are believed to play a role in maintaining tissue integrity in the fly embryo by depositing a lattice of extracellular matrix around it (149). This may also account for the ability of macrophages to migrate freely throughout the embryo, which lack a sophisticated circulatory system as found in Vertebrates. Moreover, *Drosophila* macrophages have a scavenger activity and can endocytose a wide variety of polyanionic molecules (150, 151). A *Drosophila* scavenger receptor, dSR-C1, which is expressed on embryonic macrophages, was recently shown to participate in phagocytosis of bacteria (150, 152). In the absence of dSR-C1, *Drosophila* SL2 Schneider cultured cells showed a 25% reduction in their ability to engulf bacteria, arguing that the molecular mechanisms of recognition and engulfment of bacteria may be redundant in the fly (152).

Drosophila macrophages arise from the mesoderm in the head of the embryo and in the gnathal segments at about stage 10 of development (149). At this stage, approximately 40 precursors on either side of the head of the embryo undergo 4 mitotic divisions and give rise to approximately 700 hemocytes, or blood cells, which all belong to the plasmatocyte lineage (149). They then migrate throughout the embryo following distinct stereotyped paths (149). By late stage 11, programmed cell death has initiated and plasmatocytes that encounter apoptotic cells become phagocytic (141, 148, 149). Their ability to recognize and engulf apoptotic cells prompted Tepass and colleagues to name these cells macrophages.

We previously reported the characterization of a CD36-related receptor, Croquemort (CRQ or CROQ), which is specifically expressed on macrophages during *Drosophila* embryogenesis (145). In fact, this gene product appears at late stage 11 when programmed cell death is initiated (145). This observation and the sequence homology with CD36, a mammalian receptor previously shown to participate in phagocytosis of apoptotic cells prompted us to further study its potential role in clearing apoptotic cells. Using a combination of transfection in a heterologous system and inhibition studies, we revealed that the *crq* gene could confer the ability to recognize and engulf apoptotic murine thymocytes on cultured Cos-7 kidney cells (145). We next analyzed several deficiencies that remove the *crq* locus and showed that they had a defect in phagocytosis of apoptotic corpses by macrophages in the embryo (146). This defect could be rescued by a *crq* transgene, demonstrating that *crq* is essential for efficient phagocytosis of apoptotic cells *in vivo* (146). The severity of the defect observed, a reduction of approximately 90% of the phagocytic index, argues that the CRQ-pathway is the main event required for phagocytosis of apoptotic cells and that there will not be much redundancy in the mechanisms of clearance of apoptotic cells in the fly embryo. Interestingly, macrophages in the *crq*-deficient embryos had no defect in phagocytosis of bacteria, revealing distinct pathways for the phagocytosis of apoptotic corpses and bacteria by *Drosophila* macrophages (146). These results also demonstrated conservation in the mechanisms of phagocytosis of apoptotic cells, as various CD36-family members also participate in this process in mammals (see above). Finally, these results promoted *Drosophila* as being a suitable model system to genetically dissect the molecular mechanisms underlying phagocytosis.

5. PHYSIOLOGICAL CONSEQUENCES OF APOPTOTIC CELL CLEARANCE

Although present, the clearance of apoptotic cells is not an essential function in *C. elegans*; mutants that fail to clear apoptotic cells live to the adult stage (126). Interestingly, two recent studies in *C. elegans* have demonstrated that genes involved in uptake mechanisms of apoptotic cells may in turn accelerate apoptosis of cells not yet fully committed to die (14, 15). In double mutants with a partial loss-of-function of *ced-3*, a gene encoding the *C. elegans* homologue of caspase-3 required for programmed cell death, and loss-of-function of the cell death engulfment genes, cells were observed that appeared to be in the process of dying but yet reverted to a fully viable cell as they failed to be engulfed (14, 15). Cells in animals with partial loss-of-function of *ced-3* alone eventually proceeded through programmed cell death and were engulfed by their neighbors, thus arguing for a role of the engulfment genes in triggering full-programmed cell death in cells on the verge of death (14, 15). Several previous studies in mammals also showed that phagocytosis of apoptotic cells could trigger the induction of further programmed cell death by macrophages (11-13).

In *Drosophila*, it is not known whether mutants that specifically abolish clearance of apoptotic cells are viable. Deficiencies that remove the *crq* gene are embryonic lethal. However, this lethality is already being accounted for by other genes in the *crq* vicinity, which are also deleted. A single mutation of the *crq* gene might give us an answer as to whether phagocytosis of apoptotic cells

is essential for the life of the fly. However, *crq* may also be involved in other functions and may not allow us to address this particular question. The life cycle and development of a fly is very different than that of a worm as it undergoes profound morphological changes, in particular during metamorphosis, while developing from the pupal to the adult stages. Indeed, all tissues with the exception of the imaginal discs, which will give rise to all adult appendages, are hydrolyzed at the pupal stage. Extensive programmed cell death is observed during pupariation and CRQ has been proposed to participate in the clearance of dying cells at this stage of development as well (153). Thus, failure to clear apoptotic cells during metamorphosis may have more dramatic consequences than during embryogenesis. It is known that apoptosis does not affect macrophage differentiation in the fly embryo (149). However, whether macrophages may be able to induce apoptosis has not yet been carefully looked at in *Drosophila*. While embryos that are devoid of hemocytes carry on with an apparent normal onset of apoptosis, macrophages may be competent to kill and account for at least some apoptosis. A strict identification and quantification of all apoptotic cells at all stages of development of such embryos would certainly provide an answer to that question.

In mammals, the great complexity and the redundancy in the mechanisms of clearance of apoptotic cells almost certainly reflect its many outcomes and implications, as well as its physiological importance. The use of a particular combination of receptors and ligands might indeed determine the nature of the responses triggered by the engulfment of apoptotic corpses. The dogma is that apoptotic cells are taken up before they release any cytotoxic and antigenic content (1, 10). Studies showed that failure to dispose of apoptotic cells could lead to neurodegeneration or contribute to autoimmune diseases (19, 21-23). In most cases, the uptake of apoptotic cells is accompanied by anti-inflammatory signals, such as the production of transforming growth factor-beta (TGF-beta), prostaglandin E2 (PGE2), platelet-activating factor (PAF), and interleukin-10 (IL-10) among others (16, 154-156). The synthesis and secretion of various chemokines such as the macrophage inflammatory protein-2 (MIP-2), KC and Mip-1alpha by macrophages that had engulfed apoptotic cells were inhibited, while others such as the macrophage chemotaxis protein, MCP-1 were not affected (156). These tight regulations of cytokines and chemokines most likely account for the resolution of inflammation and the regulation of immune responses.

However, an *in vitro* assay recently set up to study phagocytosis of apoptotic cells by rat microglial cells has led to rather different observations (157). Microglial cells could distinguish and engulf apoptotic rat thymocytes, as well as apoptotic encephalitogenic myelin-basic protein (MBP) T-cells. The latter are commonly used in experimental autoimmune encephalomyelitis studies that model the demyelinating disease multiple sclerosis (158). Using this assay, Magnus and colleagues showed that microglial cells that encountered apoptotic cells secreted lower amounts of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-12 (IL-12), with no changes in anti-inflammatory cytokine production, such as TGF-beta and IL-10 (159). MER, the tyrosine kinase receptor involved in the recognition of apoptotic ROS by RPE cells also inhibited tumor necrosis TNF-alpha cytokine production (160). Furthermore, while pretreatment with the tumor necrosis factor-alpha (TNF-alpha), a cytokine produced by Th1cells, or the Th2-type cytokine, TGF-beta, did not significantly affect phagocytosis of apoptotic thymocytes by microglial cells, the uptake of apoptotic cells by interleukin-4 (IL-4)-treated microglial cells was decreased by about 50% (157). Together, these results suggest a tight regulation of various cytokines that may control the inflammation, restrict auto-inflammation and minimize damage in inflamed tissues of the CNS. However, in some circumstances, clearance of apoptotic cells may also lead to macrophage activation and trigger the production of pro-inflammatory cytokines, which may become important at a wound site where more phagocytes would be needed to remove apoptotic and necrotic cells, as well as pathogens (17, 18).

Strikingly, only a small number of apoptotic corpses are seen in microglial cells at any given time, while they are expected to have an extensive phagocytic role *in vivo*, due to high levels of programmed cell death in the CNS. Interestingly, pre-incubation with interferon-gamma (IFN-gamma) allowed microglial cells to ingest more apoptotic thymocytes and faster, suggesting a role for IFN-gamma in their recruitment, as well as in the enhancement of their phagocytic activity (157). Little is currently known about how macrophages are recruited to the site of apoptosis, and whether dying cells might stimulate the recruitment of macrophages themselves. The macrophage colony-stimulatory factor, M-CSF, was recently shown to play an important role in the recruitment and differentiation of macrophages that scavenge effete cells in the uterus (161). Indeed, osteopetrotic (op/op) mice that lack M-CSF had fewer macrophages recruited in the uterus, where the endometrial epithelial cells, which secrete M-CSF, normally undergo programmed cell death and are cleared by macrophages (161). These mice also showed a defect in clearance of apoptotic epithelial cells (161).

PS ligation induces the release of TGF-beta, and the PS-receptor has been proposed to play a crucial role in switching on or off the synthesis of pro- or anti-inflammatory cytokines, depending on the particle cleared by macrophages (155). Interestingly, amastigotes of the parasite *Leishmania* spp, which are responsible for disease propagation, were shown to inhibit macrophage activity by exposing PS at their surface, a mechanism that allows the parasite to evade its killing by macrophages (24). The secretion of TGF-beta and IL-10 was also observed following *Leishmania* infection, which was dependent on PS-receptor engagement with PS, and sustained *Leishmania* growth (24). PS exposure also accounted for the uptake of the parasite (24). Thus, the parasite enters macrophages by routes that are similar to that taken by

apoptotic corpses, and subverts the ability of macrophages to participate in its killing, favoring its growth and propagation. Similarly, the pathogenic trypanosome, *Trypanosoma cruzi* (*T. cruzi*) infects macrophages and was also shown to induce the production of TGF-beta, as well as prostaglandins and polyamines. This down-modulates the ability of macrophages to trigger an appropriate inflammatory response that would lead to *T. cruzi* killing and promotes its growth in a favorable and nutritious environment (25). Many receptors that participate in the clearance of apoptotic corpses have also been shown to participate in phagocytosis of microorganisms, in particular bacteria (see above), which may thus also evade the immune system. Understanding the molecular mechanisms of apoptotic cell clearance will further our knowledge of phagocytosis of bacteria, which could also become critical in developing novel approaches to fight infectious diseases.

Finally, the role of DCs in phagocytosis of apoptotic cells, which leads to cross presentation of self-antigens, has initiated a new line of investigation with therapeutic potential in cancer vaccination. Tumor cells can evade the immune system by various means, including the induction of expression of immunosuppressive cytokines, the down-regulation or constitutive expression of pro-apoptotic genes such as Fas and its ligand, and/or the reduced expression of MHC complexes (1, 162). In two reports, DCs were fed with apoptotic or necrotic tumor cells and presented self-antigens derived from these apoptotic cells that stimulated *in vitro* or *in vivo* cellular-mediated cytotoxic immune responses, thus conferring protection against further tumor cell development in mice (26, 27). In contrast, the horizontal transfer of an activated oncogene into phagocytes that had taken up apoptotic cells expressing this oncogene was also observed, and phagocytes that received this oncogene developed a tumorigenic phenotype characterized by *in vitro* loss of contact inhibition and *in vivo* proliferative advantage (28). This result suggests that phagocytosis of apoptotic cells may occasionally be at the origin of cancer development.

6. PERSPECTIVES

Numerous receptors on phagocytes and a few apoptotic cell-ligand that participate in phagocytosis have been characterized, yet we still have a limited understanding of their relationship to each other. Professional and amateur phagocytes often employ similar receptors in the recognition and uptake of apoptotic cells, yet their efficiency of engulfment is strikingly different, and what makes a professional phagocyte such an efficient housekeeper as opposed to an amateur phagocyte remains elusive. A possible explanation for this difference could be the nature of their membrane or its fluidity, or simply the ability of professional phagocytes to migrate and travel much greater distances than an amateur phagocyte, most of which are resident cells. To date, we know very little about what triggers migration of professional phagocyte to the site of programmed cell death, and whether apoptotic cells signal to the phagocytes at a distance or whether a fortuitous proximity of the two cells is needed for the phagocyte to sense the 'eat me signals'. Such studies would provide further clues as to what the nature of apoptotic signaling molecules might be.

Most, if not all apoptotic cells share a common feature, the exposure of PS at their surface. This could simply reflect a common and quick need for all apoptotic cells to signal to phagocytes that they must be removed while ensuring an efficient and 'silent' removal of the apoptotic bodies, i.e. by triggering anti-inflammatory signals. However, PS and its receptor alone may not account for all clearance of apoptotic cells, and the outcome of phagocytosis of apoptotic cells might be directed by the presence of other phagocyte receptors and the recognition of their ligands at the surface of apoptotic corpses, thus informing the phagocyte about the nature of the cell which has died.

The physiological and pathological consequences of phagocytosis of apoptotic cells or its failure are numerous. With a renewed interest for the study of phagocytosis of apoptotic cells, we are entering a new era of investigation of the molecular mechanisms that govern a number of these important processes, such as homeostasis, tissue remodeling or shaping and the maintenance of organ functions during development, wound healing and innate immunity, autoimmunity and possibly cancer.

The identification of seven *C. elegans* genes and that of their counterparts in mammals illustrate an evolutionary conservation in the molecular mechanisms by which apoptotic cell clearance occurs. Studies of the *crq* gene in *Drosophila* show that the molecular mechanisms of apoptotic cell clearance were also conserved in insects. It is likely that studies in *C.elegans* and *Drosophila*, and the use of their powerful genetics will continue to provide valuable information on these mechanisms that will lead to a better understanding of phagocytosis of apoptotic cells in mammals.

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Abbreviations: *C. elegans*: *Caenorhabditis elegans*, DC: dendritic cell, SR-A: scavenger receptor of class A, ABC1: ATP binding cassette transporter 1, CD14: cluster of differentiation 14, PS-receptor: phosphatydilserine receptor, SP-A: surfactant protein A, CRT: calreticulin, cC1qR: collagenous tail binding C1q receptor, LDL: low density lipoprotein, LRP: LDL receptor-related protein, alpha2-m: alpha2-macroglobulin, CR3: complement receptor 3, PS: phosphatidylserine, SRs: scavenger receptors, LPS: lipopolysaccharide, CRD: carbohydrate recognition domain, RPE: retinal pigmented epithelium, ROS: rod outer segments, RCS rat: Royal College of Surgeons rat, CNS: central nervous system, APC: antigen-presenting cell, MHC: major histocompatibility complex, LOX-1: lectin-like oxidized LDL receptor, VN: vitronectin, SR_B1: scavenger receptor class B type 1, TSP-1: thrombospondin-1, beta2GPI: beta2 glycopreotein I, GAS6: growth arrest specific gene 6, MBL: mannose-binding lectin, SLE: systemic lupus erythrematosus, CED: *C. elegans* death gene, DTC: distal tip cell, SREC: scavenger recepor found on endothelial cells, PTB: phosphotyrosine binding, GULP: engulfment LRP-related protein., MBC: myoblast city, PH domain: pleckstrin homology domain, dSR-C1: *Drosophila* scavenger receptor class C type 1, CRQ or CROQ: Croquemort, TGF-beta: transforming growth factor-beta, PGE2: prostaglandin E2, PAF: platelet-activating factor, IL: interleukin, MIP-2: macrophage inflammatory protein-2, MCP: macrophage chemotaxis protein, MBP: myelin-basic protein, TNF-alpha: tumor necrosis factor-alpha, IFN-gamma: interferon-gamma, M-CSF macrophage colony-stimulatory factor, op: osteopetrotic, *T.cruzi: Trypanosoma cruzi*

Key Words: Phagocytosis, Apoptotic cells, *Drosophila*, *C. elegans*, Mammals, Professional Phagocyte, Amateur Phagocyte, Review

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