

Review Article

EFFEROCYTOSIS IN HEALTH, ILLNESS AND BEYOND: A NARRATIVE REVIEW

ABSTRACT

Our bodies generate many structures that are eventually destroyed, such as the webbing between digits in a fetus, nerve cells that cannot identify target cells during development, and cells that are recruited during an immune response. Our bodies constantly replace their cells. The human body undergoes 10^9 cell apoptosis on average per day; these cells need to be eliminated to stop their harmful contents from leaking out. (1) The primary phagocytic cell that carries out this crucial apoptotic cell clearance activity is the macrophage. (2) The term "efferocytosis," which means "to take to the grave" or "to bury," refers to this removal of dying or dead cells. (3)

INTRODUCTION

Efferocytosis is a multi-step process in which phagocytes recognize, bind, consume, and digest dying cells. A big fluid-filled vesicle holding the dead cell is formed as a result of the phagocytic cells' cell membrane expanding and engulfing the apoptotic cell throughout this process. An efferosome is the more common term for this swallowed vesicle. Efferocytosis most likely results in the removal of dead cells before their membrane integrity is compromised and their contents seep into the surrounding tissue. As a result, the surrounding tissue is shielded from harmful oxidants and enzymes and is unable to release specific intracellular components like caspases and proteases. (4)

Efferocytosis is carried out by a variety of cell types, including fibroblasts and epithelial cells, in addition to macrophages and dendritic cells. The synthesis of mediators that encourage the replacement of the apoptotic cells, such as hepatocyte and vascular endothelial growth factor, facilitates the process.

It has been determined that several efferocytic receptors, such as TIM4, bind directly to phosphatidylserine (PS) on the surface of apoptotic cells. Integrins such as $\alpha\beta3$ or $\alpha\beta5$ (6) attach to MFG-E8, (7) a soluble protein that connects the phagocyte to the apoptotic cell. IgM molecules may occasionally attach themselves to apoptotic cells

by identifying unidentified epitopes. Furthermore, this structure can be bound by C1q, which mediates complement recognition of the dying cell. The engulfment of the apoptotic cell can be facilitated by the complement system and Fc receptors, but their involvement in efferocytosis seems to be minor.

While efferocytosis and phagocytosis have many similarities, they use distinct downstream signaling pathways. Because the huge apoptotic cell is absorbed along with extracellular fluid, it also closely resembles macropinocytosis. As a result, an extremely roomy phagosome (efferosome) forms. (4)(8)(9) But in the case of phagocytosis, a tight contact between the particle and plasma membrane during engulfment results in a snugly fitting phagosome in the target. This discrepancy might be brought about by the efferocytosis-conducting activity of GTPases.

The ability of the phagocytic cell to engulf the apoptotic cell is made possible by a number of efferocytotic receptors, Fc receptors, Rac1, and Cdc42. Furthermore, it has been discovered that statins, which increase efferocytosis, inhibit Fc receptor-mediated phagocytosis. This suggests that, in addition to Rac1 and Cdc42, the two engulfment routes are alternately controlled. This could be due to the differential activation of RhoA, a GTPase that is known to oppose Rac1-mediated efferocytosis.(10) Following engulfment, the efferosome forms similarly to phagosomes, despite the observation of efferocytosis-specific proteins promoting Rab conversion on the efferosome; at last, lysosomal enzymes kill the apoptotic cell in a matter of 30 to 60 minutes.

The distinction between phagocytosis and efferocytosis can also be made based on how the dying cells interact with the macrophages. While efferocytosis is either immunologically quiet or anti-inflammatory, phagocytosis causes a pro-inflammatory reaction. The macrophages begin an anti-inflammatory program after engulfing the dead cells. A signaling cascade that involves the activation of PPAR δ (11) (12) takes place, suppressing pro-inflammatory cytokines while simultaneously inducing the strong production of cytokines that promote wound healing (such as transforming growth factor (TGF)- β) and anti-inflammatory (like IL-10). In particular, when TGF β and PGE2 are generated, the production of TNF, IL-12, and IL-1 β is down-regulated. Thus, TNF-induced inflammation in particular prevents efferocytosis. Because these antigens are self-derivative, when T- and B-lymphocytes—two members of the adaptive immune system—encounter them presented by macrophages, anti-inflammatory cytokines tell them to tolerate the antigen and any cell expressing it. Efferocytosis produces these effects by inhibiting the adaptive immune response, enhancing immunological tolerance, and promoting wound healing and remodeling.

Removing dying cells from multicellular tissues is not a novel idea. Actually, Metchnikoff explored this concept over a century ago when he spoke of "physiological inflammation," which is the process by which dead cells are removed from healthy tissues.(13) Research on nematodes revealed that cells might swallow their dead neighbors without causing inflammation, hence the word for this phenomena has to be changed. (14) (15)

Giulio Bizzozero (16), an Italian pathologist, and Marc Armand Ruffer, a physician and bacteriologist, have both made important contributions to the study of phagocytosis. They are both recognized for having written the earliest accounts of efferocytosis. Aimée deCathelineau and Peter Henson coined the term "efferocytosis" in 2003. The phrase "phagocytosis" was coined to describe the process of taking away or burying cells. The neologism was taken from the Latin prefix "effero," which means to transport, put away, or bury.

The phenomenon of programmed cell death that takes place in multicellular organisms is known as apoptosis. A few specific metabolic processes result in the cell's final morphological alterations and death. Blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA degradation are among the alterations that take place. Every day, 60 billion cells perish as a result of this process. During an organism's lifecycle, apoptosis is a critical process that is strictly regulated and controlled. In contrast to necrosis, apoptosis results in the production of specific cell fragments known as apoptotic bodies, which are swiftly removed by phagocytic cells before the hydrolytic enzymes leak out and harm nearby cells. (17) It can be started using either of the two routes. A cell that uses the intrinsic pathway kills itself when it experiences distress, whereas a cell that uses the extrinsic pathway kills itself in response to signals from other cells. The activation of particular proteases called caspases, or initiator and executioner caspases, leads to cell death and disintegration.(20) These proteins degrade the cell's substructure. Caspases then trigger enzymes that break down DNA. Under a microscope, the cell is clearly going through self-destruction at this moment. Its form varies to correspond with internal metabolic changes. The cytoplasm contracts as the cell skeleton disintegrates. Additionally, the nucleus gradually shrivels and disintegrates.

A cell's surface has docking sites, which are sensitive to signals that might shift the internal chemistry of the cell in favor of apoptosis. Tumor necrosis factor alpha (TNF α) carries one signal. T cells and activated macrophages are immune system cells that produce this signal. TNF α is not a very powerful inducer of cell death, but it can contribute to apoptosis and subsequent immunological activation.(21)

In addition to its essential function as a biological phenomena, a number of diseases have been linked to specific apoptotic process defects. While insufficient apoptosis leads to unchecked cell proliferation, as in cancer, excessive apoptosis causes atrophy. Apoptosis is induced by certain factors such as caspases and Fas receptors (22) and 23; on the other hand, several proteins belonging to the Bcl-2 family suppress apoptosis. (24)

PATHOGENESIS

Despite a lack of clarity on the precise process of pathogenesis, several theories have been proposed. The most trustworthy one claims that actin cytoskeleton rearrangement and RhoA, one of the Rho family GTPases, are required for the engulfment of the cell. Actin protrusions are formed by the macrophage around the cell, and these fuses lead to total engulfment. The resultant intracellular vesicle, known as a phagosome, starts off with a milieu that resembles the external milieu. The process of phagosome maturation involves the gradual fusing of endosomes and lysosomes driven by Rab GTPase. Then, these tiny organelles explode, releasing the enzymes that are catabolic and hydrolytic. The swallowed cell is destroyed by the phagolysosome that results, which keeps becoming more acidic until it reaches a pH of almost 4. Once an apoptotic cell is engulfed, the intracellular signaling further diverges, and the receptors that identify an apoptotic cell and facilitate efferocytosis are different from those that facilitate phagocytosis. A large phagosome known as an efferosome forms around the freshly ingested apoptotic cell during efferocytosis, when RhoA activity is inhibited and Rac1 (27) activity synchronizes the engulfment of the apoptotic body. Hydrolytic enzymes are delivered to the maturing efferosome via sequential lysosome fusion events, which is comparable to phagocytosis, even though additional GTPases are being discovered that play a role in efferosome maturation. This, together with its slow acidification, finally creates unfavorable conditions that kill the apoptotic cell.

The apoptotic cell initiates the highly regulated process of efferocytosis. Phosphatidylserine (28) is exposed to the phospholipid membrane's exofacial leaflet as an early step in the apoptotic process. The production of chemokines (29) by the dying cell attracts macrophages to the site of dying cells, which is another early occurrence. As a reaction, the macrophage upregulates the expression of tethering receptors and bridge molecules. Ultimately, the macrophage consumes the dying cells and produces the huge, roomy efferosome. Since efferocytosis produces IL-10, TGF- β , and PGE2 when macrophages consume apoptotic cells, it is thought to be an anti-inflammatory mechanism. As a result, efferocytosis is essential for the resolution of inflammation because it facilitates wound healing and tissue repair in addition to

eliminating dead cells, relieving tissue congestion, and preventing the release of phlogistic cellular contents.

The apoptotic cells generate several signals that have been loosely dubbed "apoptotic cell-associated membrane patterns." Many receptors, such as the PRRs involved in the removal of germs, mediate their identification by phagocytic cells. However, *Drosophila* embryonic macrophages express particular receptors, such as Croquemort (30). A number of genes have been linked to the phagocytosis of corpses in *Caenorhabditis elegans*, and analogs and homologs have also been found in humans.

Moreover, macrophages exposed to apoptotic cells or lysophosphatidylcholine—a particular phospholipid generated and released by dying cells—also quickly activate AMP-activated kinase (AMPK) (31). In addition to requiring calcium mobilization and the creation of certain mitochondrial reactive oxygen species, AMPK activation can also arise from the suppression of mitochondrial oxygen consumption and ATP synthesis. Following activation, there is a rise in chemokinesis and microtubule production, which subsequently adjust to the high energy requirements of engulfment and tracking. When mice were used in the experiments, lysophosphatidylcholine enhanced the absorption of apoptotic cells in the lungs. Moreover, in mice given dexamethasone, suppression of AMPK reduced the removal of apoptotic thymocytes.

Thus, the mitochondrial AMPK axis functions as a sensor and improver of apoptotic cell tracking and elimination, which is essential for tissue homeostasis and the resolution of inflammatory situations.

There are numerous levels at which efferocytosis is regulated. Apoptotic cells first release soluble substances, such as ATP and UTP, that draw macrophages to the affected area (also known as "find me" signals). These factors include the 29 chemokines CXCL1, CXCL14, CCL2, CCL6-8, and CCL11. PS, or the "eat me" signal, is a telltale indicator of programmed cell death and is found on the outer layer of the plasma membrane of an apoptotic cell. On the inner layer of the plasma membrane of healthy cells, however, PS is present. In response to the "find me" signals, macrophages upregulate bridging molecules and cell surface receptors. The cell surface receptors on PS and macrophages are bound by the bridging molecules. The macrophages utilize multiple cell surface receptors to directly identify PS, and additional receptors that attach to bridging molecules are utilized to indirectly bind PS from the apoptotic cell to the macrophage. For instance, efferocytosis is facilitated by direct bindings to PS by the PS receptor (PSR), brain angiogenesis inhibitor 1 (BAI1) (32), T cell immunoglobulin and mucin (TIM)-4, and Stabilin-2 (33). Masculine-Fat Globule Epidermal growth factor-like 8 (MFG-E8) is a bridging ligand that binds to

integrins $\beta v\beta 3$ and $\beta v\beta 5$, which are expressed on macrophages, and PS on dying cells. Tyro3, Ax1, and MerTK receptors bind to the bridging ligands Growth arrest specific-6 (Gas6) and protein S (ProS1). (34) (35) When the bridging molecules interact with the macrophages and dying cells, they cause cytoskeletal alterations that are dependent on Rac1, which facilitates efferocytosis.

Phagocytes utilize a variety of receptors that are employed by macrophages to recognize apoptotic cells. For example, MFG-E8, the PS-bridging ligand for integrins $\alpha V\beta 3$ and $\alpha V\beta 5$, is required in the mammary gland and in the retina for clearance of PS-labelled cells, while MerTK is employed in retinal pigment epithelial cells (RPECs) and mammary epithelial cells (MECs) to swallow PS-flagged cells. Macrophages' ability to efferocytose requires MerTK. Actin protrusions allow the dying/apoptotic cell to be completely engulfed by actin once MerTK is engaged by the dying cell via Gas6 or ProS1. MerTK then goes through dimerization, tyrosine kinase activation, and tyrosine phosphorylation, activating CRKII/DOCK180/ELMO signaling to the Rac GTPase. The cell is broken down by lysosomes after use. MerTK signaling not only facilitates the uptake and elimination of the apoptotic cell but also stimulates the production and discharge of immune-suppressive cytokines (IL-10, IL-13, IL-4, and TGF β 1) and inhibits the production of pro-inflammatory cytokines (IL-12, IFN). Tissue healing and immunological tolerance are improved by this.

EFFEROCYTOSIS AND IMMUNITY

Although efferocytosis is thought to have anti-inflammatory properties, more recent research has shown that some complicated responses enable the immune system to respond to infected apoptotic cells while still preserving tolerance to host molecules. When anything contributes to t-cell activation and is linked to MAMPs—microbe-associated molecular patterns—it is considered immunogenic (36). On the other hand, dying cells do not cause this kind of reaction during sepsis, therefore it is doubtful that MAMP activation is the sole deciding factor.

Dendritic cells, another type of highly phagocytic cells that are always on guard and looking for antigens in their environment, provide an additional perspective on this. .. By absorbing apoptotic blebs from infected cells and then boosting the protective T cells, they can identify antigens from microorganisms.

Similar findings were reported by Yrlid et al. (37), who discovered that pathogenic Salmonella causes infected macrophages to undergo apoptosis and that bacterial

antigens are cross-presented onto MHC class I molecules by dendritic cells, thereby stimulating CD8⁺ T cells.

Protective immunity is conferred by the apoptosis of *Mycobacterium tuberculosis*-infected macrophages and the cross-presentation of apoptotic blebs.

In a different investigation, mice were immunized with vesicles that were isolated from BCG-infected macrophages and included bacterial antigens. By using this tactic, mice developed immunity that shielded them from virulent *Mtb* infections.

A calcium-dependent binding to negatively charged phospholipid membranes is facilitated by annexin1, (38) a member of the annexin protein superfamily. A variety of functions are conferred by the N-terminal region of annexin 1. This protein aids endothelial cells in identifying, attaching to, and ingesting apoptotic cells. Furthermore, by managing T cell activation, it has been demonstrated to improve adaptive immunity.

EFFEROCYTOSIS IN OTHER DISEASES

Other diseases like cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis, and atherosclerosis have also been linked to the phenomenon of efferocytosis. (39)

ATHEROSCLEROSIS

Apoptosis is linked to the advancement of atherosclerosis, and the degree of apoptotic cell death determines the stage of the lesion and plaque rupture. Although the abundance of free apoptotic cells in the lesions points to either enhanced apoptotic death or impaired efferocytosis, the presence of macrophages holding apoptotic material implies the occurrence of efferocytosis. Numerous in vitro investigations present diverse examples demonstrating that compromised efferocytosis is a primary factor in severe atherosclerosis. It is now established that abnormalities in molecules such as TG2, MFG-E8, C1q, Mertk, lysoPC, and Fas ligand that are either directly or indirectly involved in the regulation of efferocytosis facilitate the accelerated development of atherosclerotic plaques (40).

LUNG CONDITIONS

1. Chronic obstructive pulmonary disease (COPD) exhibits the highest amount of apoptotic cells that have been maintained. According to Hodge et al., individuals with

COPD who have malfunctioning CD31, CD44, and CD91 receptors exhibit reduced efferocytosis. This illness also results in decreased expression of efferocytotic proteins such pentraxin.(39)

2. **CYSTIC FIBROSIS:** Defective efferocytosis can result from the loss of receptor recognition by Elastase.(41)

3. **IDIOPATHIC PULMONARY FIBROSIS:** In these patients, the expression of TRAIL, a protein that triggers neutrophil apoptosis, was low and the amount of uningested apoptotic cells was elevated. (42)

AUTOIMMUNE DISORDERS

Autoantigens are eliminated as a result of apoptotic cell clearance, which effectively suppresses the immune system and averts autoimmunity. The primary signal that triggers efferocytosis is the identification of PS on apoptotic cells. The synthesis of anti-inflammatory molecules like TGF- β and IL-10 is also connected to this. Auto antigens are retained when apoptotic cells are not identified and processed for destruction. This leads to the production of autoantibodies and the subsequent development of autoimmune diseases such as systemic lupus erythematosus.(43) Research carried out on mice has revealed a correlation between autoimmune and PS receptor loss of function.

EFFEROCYTOSIS AND CANCER

Within the field of cell biology, MERTK, a member of the TAM receptor tyrosine kinases, performs a variety of intricate and varied roles. Age-dependent autoimmunity caused by MERTK deficiency is typified by apoptotic cell clearance failure. Additionally, typical oncogene pathways leading to cell transformation in cancer are driven by overexpression of MERTK. In order to comprehend the relationship between efferocytosis and cell transformation, MERTK was expressed in human MCF10A cells, a non-tumorigenic breast epithelial cell line that lacks endogenous MERTK. While MERTK-10A cells did not establish persistent colonies in soft agar or exhibit higher proliferation when compared to parental MCF10A cells, their stable expression of MERTK in MCF10A resulted in enhanced motility and AKT-mediated chemoprotection (44). In addition to increasing functional capacity, MERTK induced efferocytosis. But in contrast to AXL, MERTK activation required a large percentage of apoptotic cells, indicating that MERTK might interact with phosphatidylserine preferentially.(28) Therefore, the elimination of MERTK in MDA-MB breast cancer cells decreased efferocytosis, but either temporary or stable MERTK expression enhanced the clearance of apoptotic cells in all investigated cell lines. Furthermore,

soluble TAM receptors were able to inhibit efferocytosis at higher levels in human breast cancer cells that have increased endogenous MERTK. Lastly, PD-L1 expression triggered by apoptotic cells via MERTK revealed that cancer cells could use MERTK-driven efferocytosis as an immune suppression mechanism for their own benefit. All of these findings pointed to MERTK as a crucial mediator of the development of cancer and efferocytosis, as well as a potentially undetected tumor-promoting process in the case of MERTK overexpression in epithelial cells.

Tumor growth and metastasis are caused by a reduction in CD8+ T-cells in tumors where MerTK is overexpressed. It has also been suggested that regulatory T cells, which typically regulate the activation of B and T lymphocytes, are involved in the development of cancer. TGF β is known to affect T regulatory cell production and function. TGF β is significantly elevated during efferocytosis, which contributes to the elevation of T regulatory cell numbers.

Naturally occurring killer (NK) cells (45) exhibit strong cytotoxic properties. These NK cells' expression of MerTK prevents them from developing into cytotoxic states. Numerous studies have shown that natural killer (NK) cells are antitumorous, particularly in cases of skin, breast, and colon cancers. They do this by preventing tumor initiating cells from establishing themselves and by communicating through efferocytosis receptors. In dendritic cells, Tyro3 and Axl activate suppressors of cytokine signaling, which increases the expression of anti-inflammatory cytokines while decreasing the expression of pro-inflammatory cytokines. As a result, a potent immunosuppressive reaction is present, akin to that observed during MerTK-mediated macrophage efferocytosis.

Malignant development is also aided by host immune suppression. Myeloid derived suppressor cells (MDSCs) are immature myeloid cells that are immunosuppressive and respond to infection or stress in order to preserve homeostasis. IL-10 and VEGF, which are produced in response to efferocytosis, are two examples of the substances that regulate and control MDSCs. MDSCs penetrate tumors, encourage vascularization, and obstruct the immune system's reaction to tumor antigens.

Utilizing the urokinase receptor, which is thought to be associated with the aggressiveness of different carcinomas, was the subject of another investigation. Numerous cell types express the urokinase receptor (uPAR) (48) (49) on their surface, which acts as a binding protein for vitronectin to promote cell adhesion and coordinates plasmin-mediated cell surface proteolysis for matrix remodeling. Through the utilization of both transient gene transfer and stable cell lines overexpressing uPAR, evidence was presented indicating an unreported role of uPAR in the

phagocytosis of apoptotic cells, also known as efferocytosis. Stronger apoptotic cell efferocytosis was seen when uPAR was produced in human embryonic kidney cells, hamster melanoma cells, or breast cancer cells (BCCs). It is possible that uPAR improves detection of one or more determinants on the surface of the apoptotic cell because the uPAR-expressing cells were unable to trigger the engulfment of viable cells. Mutant $\beta 5$ integrin expression did not hinder uPAR-mediated engulfment, nor did phosphatidylinositol-specific phospholipase C's cleavage of uPAR affect $\alpha v\beta 5$ integrin-mediated engulfment. Additionally, it was discovered that the more aggressive BCCs had a greater potential for phagocytic activity, which was correlated with uPAR expression. In MDA-MB231 BCCs, cleavage of membrane-associated uPAR dramatically reduced phagocytic activity. Since the resolution of inflammation and the generation of anti-inflammatory cytokines depend on efferocytosis, the overexpression of uPAR in tumor cells created a tolerogenic milieu that facilitates the growth of tumors.

Another investigation found that CD47 was used.⁽⁵⁰⁾ Acute myeloid leukemia, bladder cancer, and non-Hodgkin's lymphoma are among the human cancers that have high expression levels of CD47, which inhibits the innate immune system's ability to phagocytose cancer cells. Normal cells were largely unaffected by the phagocytosis of cancer cells and the *in vivo* eradication of tumors caused by inhibiting CD47 with a monoclonal antibody. It was therefore hypothesized that cancer cells would also need to have a strong pro-phagocytic signal. These results demonstrate the balance between pro- and anti-phagocytic signals in the immune evasion of cancer and explain why calreticulin is the predominant pro-phagocytic signal on various human malignancies. It also explains why anti-CD47 antibodies specifically target tumor cells.

FUTURE DIRECTIONS

In order to prevent acute syndromes in subjects with established cancerous lesions, the research reviewed in this review may identify specific, mechanism-based targets for these lesions, given the role of efferocytosis in cancerous lesions and the emerging concepts about how defective efferocytosis can contribute to the lesions. Determining whether additional efferocytosis-related molecules are involved in advanced lesions is vital because a great number of molecules participate in the efferocytosis process. Using a candidate molecular strategy like those outlined in this review can help achieve this goal. Advanced proteomic methods may also make it possible to analyze a wider range of efferocytosis molecules objectively and more globally.

Finding methods to improve efferocytosis in advanced human malignant tumors is a crucial objective in this field of study. Improving efferocytosis will probably result in

less inflammation, necrosis, and macrophage accumulation in advanced lesions. Additionally, there would be a decrease in their spread in the majority of advanced human tumors. It would undoubtedly be crucial for the researchers working in this field because the efferocytosis mask is not revealed in other lesions.

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