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Targeting Efferocytosis in Inflammaging

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Abstract

Rapid removal of apoptotic cells by phagocytes, a process known as efferocytosis, is key for the maintenance of tissue homeostasis, the resolution of inflammation, and tissue repair. However, impaired efferocytosis can result in the accumulation of apoptotic cells, subsequently triggering sterile inflammation through the release of endogenous factors such as DNA and nuclear proteins from membrane permeabilized dying cells. Here, we review the molecular basis of the three key phases of efferocytosis, that is, the detection, uptake, and degradation of apoptotic materials by phagocytes. We also discuss how defects in efferocytosis due to the alteration of phagocytes and dying cells can contribute to the low-grade chronic inflammation that occurs during aging, described as inflammaging. Lastly, we explore opportunities in targeting and harnessing the efferocytic machinery to limit aging-associated inflammatory diseases.

1. INTRODUCTION

Inflammation is essential for the rapid elimination of invading pathogens as well as the removal of damaged cells and initiation of the wound healing response. Thus, from an evolutionary perspective, inflammation is beneficial for the organism to defend against harmful pathogens (1). However, the increase in life expectancy in humans has revealed that inflammation could become detrimental later in life as a dysregulated inflammatory response can underpin many age-associated maladies, including autoimmune, cardiovascular, and neurological diseases. It is evident that the immune system progressively becomes dysregulated with age, and mechanisms that control inflammation in younger people become less effective in older people (2). This age-associated inflammation is generally low grade and persists chronically, conceptually termed inflammaging (2). Such persistence of unwanted inflammation can lead to further tissue damage and drive a variety of age-related diseases.

Inflammaging is influenced by many factors, including an increase in senescent cells, changes in the microbiome and metabolism during aging, and environmental factors such as diet. As inflammaging is unrelated to infection, growing evidence suggest that inflammatory stimuli originate from endogenous factors, potentially released from damaged and dead cells, and the recognition of these factors by the innate immune system could trigger and perpetuate inflammation (3). As damaged and dead cells are normally swiftly removed by phagocytes through the highly coordinated process called efferocytosis, it has been suggested that alterations in the efferocytic process during aging may contribute to inflammaging (3). Indeed, approximately 200–300 billion cells are turned over daily in the human body (4) via the programmed cell death process called apoptosis. Efferocytosis removes these dying cells and is key to preventing the release of intracellular damage-associated molecular patterns (DAMPs) such as DNA as well as nuclear proteins such as high mobility group box 1 (HMGB1) from uncleared dead cells (5). Notably, these endogenous factors can promote unwanted inflammation and autoimmunity (5, 6), and the levels of these DAMPs are also elevated with age (3), supporting the idea that efferocytosis could be impaired during aging. Furthermore, efferocytosis is also important for promoting the resolution of inflammation and tissue repair, and defects in the molecular machineries of efferocytosis can progress to chronic inflammation and the development of diseases such as colitis and autoimmune and cardiovascular diseases (7, 8), all of which are linked to inflammaging (3). In this review, we discuss the impact of aging on efferocytosis and how alteration of the efferocytic process can contribute to inflammaging, as well as therapeutic opportunities for targeting efferocytosis in inflammaging.

2. EFFEROCYTOSIS

Prompt removal of apoptotic cells through efferocytosis is achieved through highly coordinated communication between apoptotic cells, phagocytes, and neighboring cells (**Figure 1**). Efferocytosis can be broadly categorized into three key phases: (*a*) the smell phase, wherein the phagocytes sense the presence of dying cells within the tissue neighborhood; (*b*) the eating phase, which involves the recognition and engulfment of dying cells by phagocytes; and (*c*) the digestion phase, in which the dying cell–derived materials are processed by phagocytes. Molecular mechanisms underpinning these efferocytic steps are described further below.

2.1. Smell Phase of Efferocytosis

Commitment to apoptosis following caspase 3/7 activation can lead to proteolytic processing of cellular substrates that are key to initiating communication between apoptotic cells and phagocytes, thus establishing the first phase of efferocytosis to signify the site of cell death. This

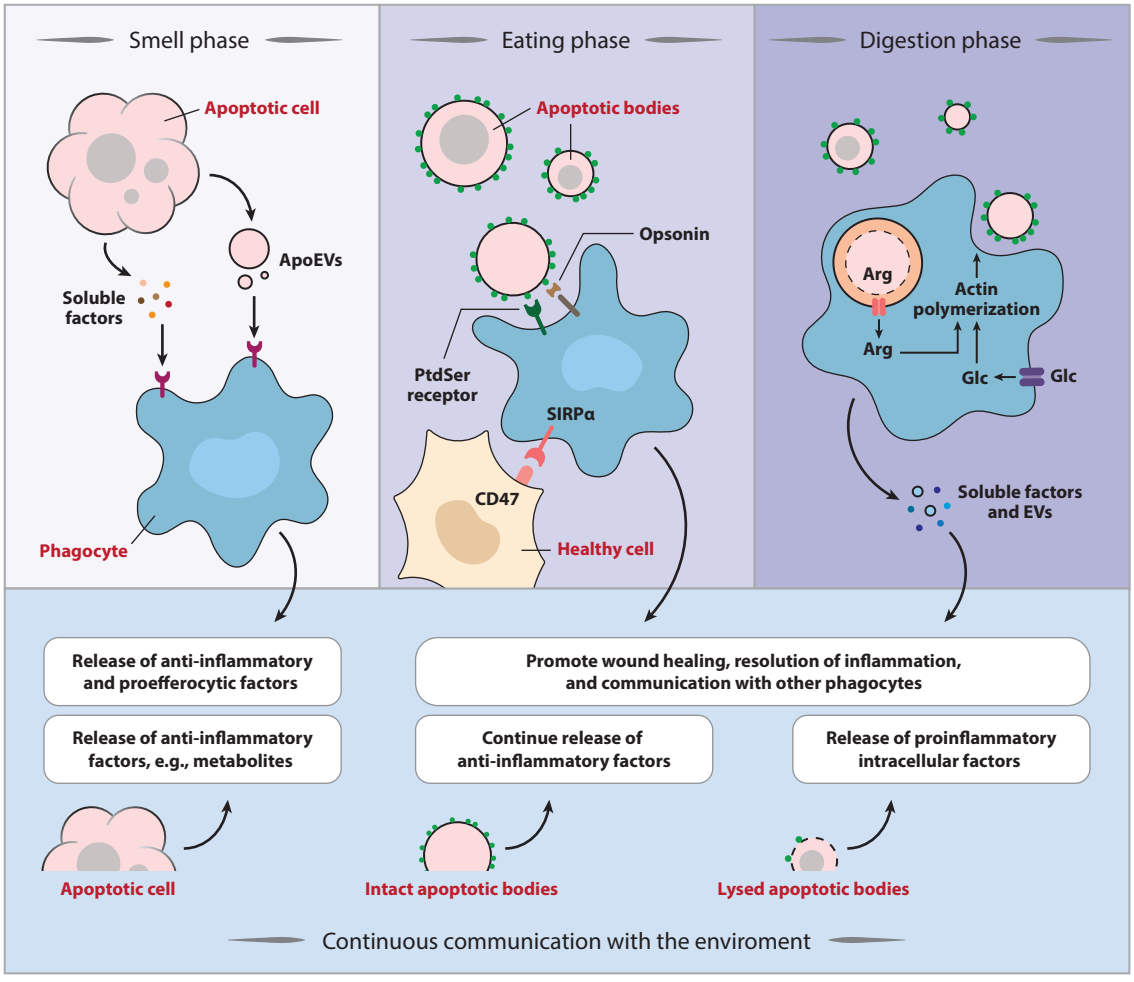

Figure 1

Efferocytosis can be distinguished into three major phases denoted as the smell, eating, and digestion phases. Cells undergoing apoptosis can release and expose a variety of molecular factors to aid the detection, recognition, and uptake of dying cells by phagocytes. Processing of apoptotic materials can also fuel further rounds of efferocytosis. During efferocytosis, dying cells and phagocytes will continuously communicate with neighboring cells to coordinate cell clearance and promote processes such as the resolution of inflammation and tissue repair. Abbreviations: ApoEV, apoptotic extracellular vesicle; CD47, cluster of differentiation 47; EV, extracellular vesicle; PtdSer, phosphatidylserine; SIRPα, signal regulatory protein alpha.

smell phase of efferocytosis can facilitate both the recruitment of phagocytes toward the site of cell death and the priming of phagocytes for later processes. First, to attract the migration of phagocytes such as monocytes and macrophages toward apoptotic cells, soluble factors, including metabolites and lipid biomolecules, which are collectively termed as find-me signals, are released from apoptotic cells (5, 9). During early stages of cellular apoptosis, caspase-activated opening of Pannexin 1 channels mediates the release of nucleotides such as adenosine triphosphate (ATP) and uridine triphosphate (UTP), which can be sensed by P_2Y_2 receptors on phagocytes to help direct their migration toward apoptotic cells (10, 11). Lipid biomolecules such as lysophosphatidylcholine and sphingosine-1-phosphate (S1P) can also be released by apoptotic cells and

function as find-me signals (12, 13). Furthermore, chemotactic factors can be associated with extracellular vesicles (EVs) released from apoptotic cells. For instance, chemokines such as CX_3CL1 and CCL12 were found on apoptotic cell–derived extracellular vesicles (ApoEVs) to promote macrophage recruitment toward dying cells (14, 15). Notably, not all types of phagocytes are recruited to aid apoptotic cell clearance. During apoptosis, dying cells can also release the so-called keep-out signal, lactoferrin, to inhibit neutrophil migration (16) as another mechanism to limit inflammation at sites of cell death.

In addition to alerting the phagocytes to migrate closer to dying cells, apoptotic cells can also release factors to upregulate efferocytic machineries in preparation for eating and digesting apoptotic materials. S1P released from apoptotic cells can activate downstream signaling from its receptor, S1PR1, on macrophages, resulting in the production of erythropoietin (EPO); activation of EPO signaling by a paracrine/autocrine mechanism can then upregulate efferocytic molecules such as Mer tyrosine kinase (MerTK), growth arrest–specific 6 (Gas6), milk fat globule factor-E8 (MFG-E8), and CD36 through peroxisome proliferator-activated receptor γ (PPARγ) activation (17). Furthermore, the secretome of apoptotic cells can upregulate gene programs related to cell motility, cytoskeletal rearrangement, metabolism, and anti-inflammatory responses in phagocytes (18). In particular, the apoptotic secretome can upregulate the molecules required for improved glucose uptake to promote efferocytosis (19). Thus, these studies suggest that the smell phase can prepare the phagocytes for later stages of efferocytosis.

2.2. Eating Phase of Efferocytosis

Following recruitment to the site of cell death, phagocytes need to distinguish apoptotic cells from other healthy cells in the surrounding tissue to ensure that apoptotic cells are removed specifically. To achieve this, apoptotic cells expose eat-me signals such as phosphatidylserine (PtdSer) to aid their recognition by phagocytes (20, 21). Phospholipids are distributed asymmetrically at the plasma membrane, and PtdSer is located predominately at the inner leaflet in healthy cells (22). To disrupt this phospholipid asymmetry during apoptosis, the scramblase XK-related protein 8 (Xkr8) is irreversibly activated by caspase cleavage to promote exposure of PtdSer on the outer leaflet of the plasma membrane (23). To maintain PtdSer on the surface of apoptotic cells, the flippase ATP11C, which facilitates the transport of aminophospholipids such as PtdSer from the outer leaflet to the inner leaflet of the plasma membrane, is also inactivated by caspase cleavage (24). Besides PtdSer, other phospholipids such as phosphatidylinositides and proteins such as calreticulin are also exposed on the surface of apoptotic cells and can function as eat-me signals (25, 26). PtdSer exposed on apoptotic cells can be recognized directly by a number of receptors on phagocytes, such as T cell immunoglobulin and mucin domain containing 4 (TIM-4), brain-specific angiogenesis inhibitor 1 (BAI-1), and stablin 2 (27–29). Engagement of PtdSer with BAI-1 and stablin 2 can subsequently activate downstream signaling through engulfment and cell motility 1 (ELMO)/dedicator of cytokinesis 180 (Dock180)/Rac and GULP/Rac proteins, respectively, to trigger cytoskeletal changes required for apoptotic cell engulfment (28, 29). Furthermore, apoptotic cells can also be recognized by phagocytes though opsonins such as MFG-E8, Gas6, and Protein S, which help bridge PtdSer on apoptotic cells and efferocytic receptors such as αVβ3/5 integrin and TAM family of receptors [i.e., tyrosine receptor kinase 3 (Tyro3), Axl, and MerTK] on phagocytes (30–32). To further aid phagocyte detection, apoptotic cells can also downregulate don't-eat-me signals such as CD47 on the cell surface, thus preventing signal regulatory protein alpha (SIRPα)-mediated signaling in macrophages that negatively regulate engulfment (26). Collectively, these molecular changes on apoptotic cells facilitate a clear distinction from healthy cells and thereby trigger the engulfment process.

2.3. Digestion Phase of Efferocytosis

Following recognition and engulfment of apoptotic cells, apoptotic cargo can be processed and degraded by phagocytes through the canonical degradation program or by other parallel pathways. The canonical degradation pathway encompasses the maturation of the early phagosome to late phagosome, with the subsequent fusion of the late phagosome and the lysosomal compartment facilitating the degradation of apoptotic materials (33). Apoptotic cargo can also be degraded through microtubule-associated protein 1A/1B-light chain 3 (LC3)-associated phagocytosis (LAP), whereby the autophagy machinery is translocated to the phagosome to form the LAPosome to process apoptotic materials (34). A critical matter to consider at this phase of efferocytosis is how the phagocytes can handle the metabolic burden following ingestion of apoptotic cells containing an abundant amount of lipids, proteins, nucleic acids, and metabolites, as detailed in several reviews (8, 35). Briefly, as a mechanism to handle excessive lipids within macrophages following efferocytosis, the cholesterol transporter protein ATP-binding cassette transporter A1 (ABCA1) is upregulated following apoptotic cell detection at the eating phase to facilitate the efflux of cholesterol to apolipoprotein A1 (36, 37). As phagocytes such as macrophages are often required to ingest multiple apoptotic cells and fragments at the site of cell death, degraded apoptotic materials can also be metabolized to fuel further rounds of efferocytosis. It was recently found that amino acids such as arginine, derived from degraded apoptotic materials, are transported to the cytosol of macrophages via the lysosomal arginine transporter proline-glutamine loop repeat-containing 2 (Pqlc2), with further metabolic processing of arginine to ornithine and putrescine by arginase 1 (Arg1) and ornithine decarboxylase (ODC), respectively, driving subsequent rounds of efferocytosis (38).

2.4. Continuous Communication with the Local Environment During Efferocytosis

At each phase of efferocytosis, apoptotic cells/fragments and efferocytosing phagocytes constantly communicate with neighboring cells to coordinate a cooperative clearance of apoptotic cells as well as promote the resolution of inflammation and tissue repair (**Figure 1**). For example, the release of metabolites such as spermidine and guanosine 5′-monophosphate (GMP) from apoptotic cells through caspase-activated Pannexin 1 channels can alter the gene programs of neighboring cells to establish anti-inflammatory and wound healing responses (18). In certain settings, apoptotic cells can also release cytokines such as transforming growth factor-β (TGF-β) (39) and IL-10 (40) to help establish an immunosuppressive microenvironment. It should also be noted that apoptotic cells can generate large ApoEVs [i.e., apoptotic bodies (ApoBDs)] and small ApoEVs (exosome- and microvesicle-like vesicles) during cell death to regulate a variety of processes through the cargo packaged within or exposed on the EVs (41). For example, ApoBDs generated from epithelial cells carrying Wnt ligand Wnt8a are able to stimulate the proliferation of neighboring stem cells (42). Unexpectedly, a number of studies have also reported the ability of small ApoEVs to harbor DAMPs and promote sterile inflammation (43, 44). Although efferocytosis is a rapid and efficient process, a proportion of apoptotic cells and/or ApoBDs may inevitably progress to membrane lysis (45). In this scenario, intracellular inflammatory factors are also exposed, highlighting that the immunological outcome of efferocytosis is controlled by a complex combination of factors even in the absence of pathogens.

From first smelling the presence of apoptotic cells to eating and digesting the dying cells, phagocytes constantly communicate with neighboring cells, in particular through the release of factors that can modulate efferocytosis, the anti-inflammatory response and tissue repair. As noted above, the release of S1P by apoptotic cells during the smell phase can promote the release

of EPO from macrophages, which can signal to other macrophages to upregulate efferocytic machineries (17). Uptake of apoptotic cells by macrophages through the activation of MerTK receptor could promote the biosynthesis and release of specialized proresolving mediators (SPMs) such as Resolvin D1 (46–48), which can in turn promote efferocytosis and anti-inflammatory response (47–49). Professional phagocytes such as macrophages that have engulfed apoptotic cells can also communicate with neighboring nonprofessional phagocytes such as epithelial cells to coordinate apoptotic cell clearance, with macrophages releasing insulin-like growth factor 1 (IGF-1) and microvesicles to redirect epithelial cells to take up small EVs (e.g., microvesicles) and dampening their inflammatory response (50). Furthermore, uptake of apoptotic cells and the consequent increase in cellular fatty acids and activation of metabolic signaling pathways can stimulate the efferocytosed macrophages to release anti-inflammatory cytokines such as IL-10 (51). Similarly, during the digestion phase of efferocytosis, certain solute carrier (Slc) transporters such as Slc16A1 are also upregulated on phagocytes to mediate the release of lactate to promote an anti-inflammatory environment (19). Collectively, these studies, as well as many other studies not described in this review due to space limitations, highlight the dynamic communication between dying cells, phagocytes, and neighboring cells at the site of cell death throughout all phases of efferocytosis to ensure appropriate appreciation of the meal and elicit suitable downstream responses. One or more of these steps involving the complex interplay between dying cells and phagocytes can go awry or be less effective with age.

3. CHANGES IN EFFEROCYTOSIS DURING AGING

Inflammaging is characterized by a chronic state of low-grade systemic inflammation and can be influenced by many factors, and sterile inflammation caused by impaired disposal of inflammatory self-molecules has been proposed as one key underpinning factor (3). Since efferocytosis is a critical process to ensure rapid removal of self-molecules in dying cells, the way in which the efferocytic machineries change during aging contributes to inflammaging is discussed below. In particular, how aging could impact the efferocytic process through changes in the phagocyte (i.e., the diner) as well as the dying cell (i.e., the menu) is explored.

3.1. Alteration of the Diner

Macrophages are an important phagocytic subset that perform efferocytosis in many tissues, and changes in the phagocytic activity of macrophages during aging have been investigated with conflicting observations. For example, earlier in vitro studies using peritoneal macrophages isolated from older mice observed an increase in phagocytic activity compared to younger mice (52). However, later in vitro studies observed an impairment in latex particle uptake by peritoneal macrophages isolated from older mice (80–90 weeks) compared to younger mice (13–17 weeks) (53). More recently, peritoneal macrophages from older mice (15–20 months) showed a defect in fluorescent particle uptake versus younger mice (2–3 months) under both in vitro and in vivo settings (54). Similarly, conflicting observations have been reported in studies using phagocytes isolated from human volunteers. $CD14^+$ monocytes from elderly individuals (67–84 years old) showed lower efficiency in phagocytosing fluorescently labeled *Escherichia coli* compared to those from younger individuals (20–34 years old) (55), while other studies did not observe significant changes in the uptake by phagocytes isolated from elderly (66–100 years old) and younger (25–45 years old) individuals (56). Despite these contradictions in age-related uptake of bacteria or synthetic targets, the negative impact of aging on apoptotic cell removal by macrophages has been more consistently observed.

The effect of aging on efferocytosis has been examined in a number of different settings using mouse models. In a model of ultraviolet radiation–induced keratinocyte apoptosis, older mice (24 months old) displayed impaired removal of apoptotic keratinocytes compared to younger mice (2 months old) (57). Whether this impaired apoptotic keratinocyte clearance occurred due to a defect in macrophage-mediated efferocytosis was not defined, but the authors also observed delayed removal of apoptotic T cells by peritoneal macrophages in older mice in vivo. Most intriguingly, the defect in cell clearance was found to be caused by serum factor(s) present in older mice, whereby peritoneal macrophages from young mice treated with serum from older mice reduced their efferocytic activities (57). The observation that the microenvironment under aging conditions could influence the phagocytic function of peritoneal macrophages is also reflected in other studies, in which older mice were found to have more B cells and elevated levels of IL-10 in the peritoneum (54), thereby limiting phagocytosis (58). Furthermore, a reduction in proresolving lipid mediators in the peritoneum of older mice (20 months old) compared to younger mice (2 months old) has also been observed when the mice were challenged with zymosan to induce peritonitis. Consequently, older mice had a delayed resolution of inflammation as measured by increased polymorphonuclear neutrophils (PMNs) and proinflammatory cytokines compared to younger mice (59). Similarly, an imbalance in proresolving lipid mediators and proinflammatory factors has also been observed in older mice when subjected to hind-limb ischemia-reperfusion injury; older mice (16 months old) exhibited defective efferocytosis and heightened inflammation compared to younger mice (3 months old) (60). Together, these studies highlight how aging could alter the microenvironment of macrophages and thereby influence their efferocytic properties.

Mechanistically, how could aging impair efferocytosis? A number of studies have shed light on the underpinning processes; in particular, alteration of the PtdSer recognition machineries in macrophages has been attributed to delayed efferocytosis in both animal and human studies of aging. MerTK, an efferocytic receptor that detects PtdSer on apoptotic cells through Gas6 or Protein S, was found to be more proteolytically cleaved in 16-month-old mice compared to 3-month-old mice in a model of remote lung injury, resulting in impaired efferocytosis of inflammatory cells and delayed resolution of inflammation in older mice (60). Interestingly, both young and old MerTK cleavage–resistant mice exhibited comparable levels of inflammation, elegantly demonstrating that MerTK cleavage in older mice underpins the exacerbated inflammatory response (60). Notably, cleavage of MerTK in older mice was suggested to be caused by the accumulation of senescent cells during aging, which can lead to an imbalance of proresolving lipid mediators and proinflammatory factors and promote MerTK cleavage (60). In another study, TAM receptors such as Axl and the opsonin Gas6 were also downregulated in bone marrow macrophages isolated from older mice (20–30 months old) compared to those from younger mice (8–16 weeks old), a process that can contribute to impaired removal of senescent neutrophils in the bone marrow and circulation of older mice (61). Furthermore, human studies using a vesicant-induced acantholysis/blister model have also observed a reduction in the expression of PtdSer receptor TIM-4 on mononuclear phagocytes in elderly individuals (>65 years old) compared to younger individuals (18–40 years old) (62). Further, elderly individuals showed an impairment in inflammation resolution and clearance of PMNs, with mononuclear phagocytes in the blister exhibiting a cell-intrinsic defect in efferocytosis. Mechanistically, the reduction in TIM-4 expression on mononuclear phagocytes from elderly individuals was found to be modulated by the p38 mitogen-activated protein kinase (MAPK)-p300 signaling axis (62). It should be noted that the aforementioned animal and human studies did not examine whether gender differences could impact efferocytosis during aging, as most animal studies only used male mice. As males and females differ greatly in their susceptibility to immunological diseases such as autoimmunity (63), whether there are sex-dependent changes in efferocytosis during aging needs to be further explored.

In many tissues, the number of cells that undergo turnover far outnumbers the number of macrophages. Thus, macrophages are often required to ingest multiple dying cells and cell fragments continuously at the sites of cell death (8). This repeated cycle of apoptotic cell engulfment is regulated by a number of mechanisms, including mitochondrial fission (64, 65). The initial interaction between the macrophage and apoptotic cell triggers dynamin-related protein 1 (Drp1)-mediated mitochondrial fission, which promotes the release of calcium from the endoplasmic reticulum to drive phagosome-lysosome fusion and vesicular trafficking, thereby enabling the macrophage to perform further rounds of efferocytosis (64). Notably, dysregulation of mitochondrial fission and fusion is observed during aging such that proteins involved in mitochondrial fission decline with age, while promoting mitochondrial fission prolongs the life span of model organisms (66). Thus, perhaps the ability of phagocytes to continuously efferocytose multiple dying cell corpses could be impaired by dysregulation of mitochondrial fission during aging. Furthermore, continued/successive clearance of apoptotic cells is also regulated by mitochondrial uncoupling protein 2 (Ucp2), and the deficiency in Ucp2 can result in impaired efferocytosis in vitro and in vivo (67). Intriguingly, a polymorphism in *UCP2* is associated with longevity in humans, and Ucp2-deficient mice have a shorter life span and exhibit early signs of aging, including early onset of age-associated inflammatory diseases such as ulcerative dermatitis (68). It is worth noting that dysregulation of mitochondrial function is one of the 12 hallmarks of aging (69) and can have broad impacts on many processes beyond efferocytosis. Nevertheless, these studies highlight a potential link between mitochondrial dysfunction and impaired efferocytosis during aging.

Besides the eating phase, aging can potentially influence other phases of efferocytosis. For example, the migration of human monocytes has been suggested to increase with age due to altered expression of proteins involved in endothelial cell attachment and transendothelial migration, such as more CD11b and less CD62L, respectively (55, 70). Notably, P_2Y_2, a nucleotide receptor that is important for phagocytes to respond to find-me signals, showed a modest increase in expression in murine microglia during aging (71). In contrast, an earlier study reported that immune cells from elderly individuals (66–100 years old) showed a defect in migration toward serum-derived chemotactic factors compared to immune cells from younger individuals (25–45 years old) (56). However, whether aging could directly affect the migration of phagocytes toward apoptotic cells is not well defined and warrants further investigation.

During efferocytosis, phagocytes can release numerous factors to promote the resolution of inflammation and wound healing response. The impact of age on the communication between phagocytes and the local environment has also been addressed. For example, a reduction in TIM-4 expression on mononuclear phagocytes in elderly individuals was found to alter downstream proresolution pathways, with the loss of TIM-4 impairing the production of anti-inflammatory cytokine TGF-β following apoptotic cell encounter, thus further exacerbating inflammation (62). It should also be noted that the uptake of apoptotic cells by macrophages can stimulate the release of factors such as IGF-1 and microvesicles to regulate the efferocytic and inflammatory properties of neighboring epithelial cells (50). Although how aging affects this phagocyte-phagocyte communication has not been examined, it is worth noting that there is ample evidence demonstrating that the quantity and composition of EVs could change during aging, with a number of studies suggesting that small EVs in the circulation are reduced during aging (72). Thus, how aging could influence the communication between diners should be explored further.

Although professional phagocytes such as macrophages get much of the attention in cell clearance and phagocytosis of other exogenous targets, the stromal cells such as epithelial cells and fibroblasts are quite capable of efferocytosis. While the latter may be less proficient in eating, they make up for their reduced capacity for efferocytosis through their numerical supremacy. Thus,

when considering the effects of age on efferocytosis, one also needs to consider the type of phagocytes and their properties. In fact, it has been reported that properties of lung epithelial cells are altered with age in both mice and humans (73, 74). Given that airway epithelial cells can engulf their dying brethren and can influence the outcome/severity of airway inflammation (50, 75), future studies need to consider both professional and nonprofessional phagocytes for age effects. Further, the responses of these phagocytes after efferocytosis may also change differently with age, and this is another variable to consider while making interpretations.

3.2. Changing the Menu

To date, most studies have focused mainly on changes in phagocyte function during aging. However, since dying cells provide many signals to aid in their clearance (**Figure 1**), it is conceivable that changes in dying cells (i.e., the menu) during aging could also impact the efferocytic process. For example, release of metabolites from caspase-activated Pannexin 1 channels can regulate phagocyte recruitment (10, 11) and establish anti-inflammatory and wound healing responses (18, 76, 77). Thus, changes in the expression of Pannexin 1 as well as enzymes in various metabolic pathways can potentially influence the efferocytic process. Indeed, changes in metabolite levels during aging have been reported in a number of studies (78). Our recent data also suggest that mice lacking Pannexin 1 have a shorter life span compared to control littermates (C.B. Medina, B. Barron & K.S. Ravichandran, unpublished observations). Furthermore, the exposure of eat-me signals could be influenced by aging, although there is no evidence to suggest that PtdSer exposure machinery is altered during aging. Notably, dying cells can be decorated with opsonins such as Gas6, complement component 1q (C1q), and histidine-rich glycoprotein (HRG) to bridge eat-me signals and engulfment receptors on phagocytes (31, 79, 80). Changes in the levels of these opsonins during aging have been described, including the increase of C1q in the brains of aged mice and humans (81), the increase of HRG in the plasma of older individuals (50–59 years old) (82), and a reduction in Gas6 expression in oocytes from older female mice (12 months old) (83). Whether aging could affect the release of apoptotic cell–derived factors and/or exposure of molecular signals on apoptotic cells to trigger their recognition and uptake remains to be determined.

Besides apoptotic cell clearance, efferocytosis is also important for the removal of membrane permeabilized cells, including necrotic cells or cells that have died through other forms of programmed cell death pathways such as necroptosis and pyroptosis (84). Although the engulfment of necrotic, necroptotic, and pyroptotic cells can be mediated through similar efferocytic machineries (85, 86), concomitant release of inflammatory factors by these types of dying cells is likely to influence the immunological outcome of efferocytosis (84). Notably, it has been reported that fibroblasts isolated from elderly individuals (60–80 years old) were more susceptible to undergoing necrosis than apoptosis when treated with hydrogen peroxide compared to fibroblasts from younger individuals (17–60 years old). This increased susceptibility of fibroblasts to undergo necrosis rather than apoptosis was due to reduced ATP levels in fibroblasts from elderly individuals (87). It is also important to note that cell death is regulated by many molecular factors, and the expression levels of cell death regulators is key to influencing which form of programmed cell death a cell will undergo (88, 89). Interestingly, the expression of apoptosis regulators was found to be altered in the skeletal muscles of rats during aging (90), suggesting that aging could potentially alter the proportion of cells undergoing different forms of programmed cell death and influence how phagocytes appreciate the meal. Furthermore, a study in the model organism *Drosophila* has described the association between aging and the gradual increase of apoptosis in muscle and fat (91). Whether an increase in cell death in a specific tissue during aging may pose a challenge for neighboring phagocytes in efficiently mediating cell clearance warrants further investigation.

Collectively, changes in the quality and quantity of dying cells during aging in specific tissue may affect the outcome of efferocytosis.

4. THERAPEUTIC OPPORTUNITIES IN TARGETING EFFEROCYTIC PROCESSES IN AGING

As many aspects of the efferocytic process can be altered during aging and contribute to unwanted inflammation, this opens new therapeutic opportunities in targeting efferocytosis to limit the chronic inflammation associated with aging (**Figure 2**). The anti-inflammatory and wound healing responses after apoptotic cell clearance also highlight therapeutic opportunities to harness efferocytosis to treat diseases associated with aging. These two therapeutic possibilities are discussed further below.

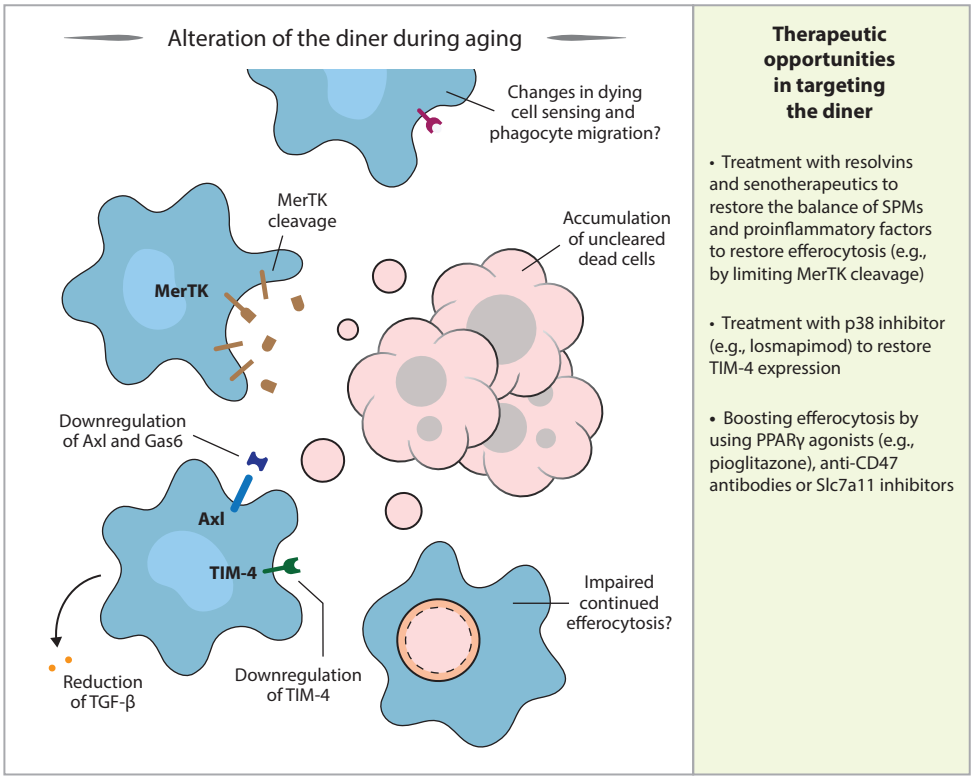

Figure 2

Alteration of the diner during aging and therapeutic opportunities in targeting the diner to restore or boost efferocytosis. (*Left*) Aging could alter the efferocytic properties of macrophages via a number of mechanisms, including the cleavage or downregulation of efferocytic receptors such as MerTK, Axl, Gas6, and TIM-4, resulting in the accumulation of uncleared dead cells. During aging, the ability of macrophages to efficiently detect dying cells and efferocytose multiple cell corpses continuously may also be altered. (*Right*) Efferocytosis can be enhanced by targeting phagocytes via approaches such as counteracting specific alterations during aging (e.g., with p38 inhibitors to restore TIM-4 expression) or by broadly boosting efferocytosis (e.g., with PPARγ agonists). Abbreviations: CD47, cluster of differentiation 47; Gas6, growth arrest–specific 6; MerTK, Mer tyrosine kinase; PPARγ, peroxisome proliferator-activated receptor γ; Slc7a11, solute carrier family 7 member 11; SPM, specialized proresolving mediator; TGF-β, transforming growth factor-β; TIM-4, T cell immunoglobulin and mucin domain containing 4.

4.1. Targeting Efferocytic Machinery

As noted earlier, disruption of the p38 MAPK signaling axis was described as the underpinning mechanism of reduced TIM-4 expression on mononuclear phagocytes from elderly individuals, resulting in impaired efferocytosis and resolution of inflammation (62). To target this impairment therapeutically, p38 blockade by losmapimod, a p38 inhibitor that is currently undergoing clinical trial, restored TIM-4 expression and efferocytic activities of mononuclear phagocytes from elderly individuals under in vitro conditions. Impressively, elderly individuals that took losmapimod orally performed better in the resolution of skin inflammation, with a reduction in uncleared PMNs and an increase in TIM-4 expression on mononuclear phagocytes at the site of inflammation (62). Notably, a number of small-molecule inhibitors of p38 MAPK have completed Phase I clinical trials with promising safety profiles (92, 93), and losmapimod is currently being tested in a Phase III clinical trial for facioscapulohumeral muscular dystrophy (NCT05397470). Thus, therapeutically targeting efferocytosis in inflammaging in humans using p38 inhibitors could be a feasible approach.

Disrupting the balance of SPMs such as Resolvin D1 versus proinflammatory factors is another contributing factor to impaired efferocytosis during aging (59, 60). Thus, these studies highlight the possibility that re-establishing the balance between SPMs and proinflammatory factors could be a potential therapeutic approach to restore efferocytosis in elderly individuals. Promisingly, in animal studies, administration of the SPM Resolvin D1 was able to enhance removal of PMNs in older mice as well as prevent cleavage of the efferocytic receptor MerTK during aging (60). Similarly, intraperitoneal delivery of specially formulated Resolvin D1 and Resolvin D3 into older mice promoted efferocytosis of PMNs and resolution of inflammation in a zymosan-induced model of peritonitis (59). Collectively, these studies suggest that resolvin-based treatment is a feasible therapeutic approach to limit inflammation in aging by promoting efferocytosis. It is interesting to note that the levels of Resolvin D1 are also reduced in a rat model of Parkinson's disease (PD) as well as in humans with PD, and administration of Resolvin D1 in rat could limit neuroinflammation and prevent the onset of neuronal and motor deficits (94). Since neurodegenerative diseases such as PD have been proposed to be associated with inflammaging (95), developing SPM-based therapeutics is an attractive approach to target multiple aspects of inflammaging. Notably, SPMs can be readily purchased as a dietary supplement. It is also worth noting that secretory factors from senescent cells were able to impair efferocytosis by disrupting the balance of SPMs and proinflammatory factors as well as promoting MerTK cleavage (60). Since the accumulation of senescent cells generally increases with age (96), using senotherapeutics to eliminate senescent cells (97) could be an indirect approach to restore efferocytosis during aging.

In addition to counteracting specific changes during aging to restore impaired efferocytosis, other pharmacological approaches are also available to promote efferocytosis (7) and may be suitable to treat pathological conditions associated with inflammaging. For example, activation of PPARγ using the PPARγ agonist pioglitazone can boost efferocytosis of peritoneal macrophages in a mouse model of chronic granulomatous disease (98), presumably though the upregulation of efferocytosis-associated molecules such as MerTK, MFG-E8, and CD36 (17, 99), as well as promoting glucose catabolism to facilitate the engulfment process (100). Since pioglitazone is already used clinically as an antidiabetic drug (101) and activation of PPARγ by PPARγ agonists has additional anti-inflammatory effects through other mechanisms (102), pioglitazone could be used to treat inflammatory diseases associated with aging (103). Furthermore, the expression of don't-eat-me-signal CD47 can be upregulated in certain cancers (104) and in atherosclerotic plaques (105). Blockade of CD47 using anti-CD47 antibodies has been shown to be effective in promoting phagocytosis to aid in the clearance of cancer cells (106) as well as apoptotic debris in

the atherosclerotic plaques (105). As the development of cancer and cardiovascular diseases (e.g., atherosclerosis) is also linked to inflammaging (107), anti-CD47 treatment is another therapeutic option to boost efferocytosis during aging. Recently, other approaches to genetically and pharmacologically enhance efferocytosis both in vitro and in vivo have been reported. In one case, pharmacological blockade of the cysteine/glutamate transporter solute carrier family 7 member 11 (Slc7a11) in skin dendritic cells potently promoted efferocytosis and improved wound healing in both control and diabetes-prone mice. Further, mice deficient in Slc7a11 also showed improved wound healing after full-thickness skin wounds (108). In another approach, the expression of chimeric efferocytic receptors, where a signaling-competent fragment of ELMO was fused to the cytoplasmic tail of either BAI-1 or TIM-4, could potently boost efferocytosis in multiple inflammatory models such as hepatitis, acute kidney injury, and colitis models (109). Whether the expression of such chimeric receptors through lipid nanoparticles or virus-like particle delivery approaches in specific inflammatory conditions might provide a benefit in resolving or dampening inflammation is an attractive idea that remains to be tested.

Besides targeting the diner, the menu can also be modulated therapeutically. For example, the disassembly of apoptotic cells into smaller fragments called ApoBDs is regulated by the formation of a thin membrane protrusion known as apoptopodia, a process negatively regulated by caspase-activated pannexin 1 (PANX1) channels (110, 111). Notably, inhibition of PANX1 promotes the fragmentation of apoptotic cells into ApoBDs, a process that could aid efferocytosis (112) and potentially promote phagocyte recruitment as ApoBDs can harbor find-me signals (15). Pharmacological inhibition of PANX1 is also quite feasible as there are a number of drugs currently being used in the clinic that are PANX1 inhibitors (113). However, blockade of PANX1 to promote cell fragmentation and engulfment must be considered with caution since PANX1 is also important for the release of metabolites that regulate phagocyte recruitment, the resolution of inflammation, and tissue repair (10, 18, 77). Furthermore, a major consequence of impaired efferocytosis is the release of intracellular factors following membrane lysis that cause unwanted inflammation (5, 104). More recently, a number of molecular factors such as gasdermin E (GSDME) and ninjurin-1 (NINJ1) were linked to mediating membrane lysis during apoptosis and other forms of programmed cell death (114–116). Thus, development of pharmacological approaches to delay the onset of membrane permeabilization by targeting these modalities could be another avenue to enable efferocytosis prior to cell lysis. Notably, GSDME-mediated cell lysis could be partially blocked by the C-Jun N-terminal kinase inhibitor SP600125 (117, 118), and NINJ1 could be inhibited by neutralizing antibodies (119–121).

4.2. Harnessing Anti-inflammatory Properties of Apoptotic Cells

Molecular signals exposed or released by apoptotic cells are well known to promote the resolution of inflammation and wound healing responses. Thus, harnessing these molecular signals could facilitate the development of therapeutics for pathological conditions that are associated with inflammaging (**Figure 3**). Apoptotic cell–based therapies have been investigated in a number of disease models, particularly chronic inflammatory conditions that are linked to inflammaging such as arthritis and colitis. For example, intravenous administration of apoptotic murine thymocytes markedly reduced the severity of collagen-induced arthritis in mice (122). Similarly, a single intravenous infusion of apoptotic murine splenocytes was able to reduce the severity of intestinal inflammation in a model of dextran sodium sulfate–induced colitis (123). Since infusion of human leukocyte antigen (HLA)-matched apoptotic cells is deemed safe and no toxicity was observed in a Phase I/IIa clinical trial (124), it is conceivable that apoptotic cells can be used to treat chronic inflammatory diseases, with a number of pharmacological companies such as Enlivex Therapeutics developing apoptotic cell–based therapeutics.

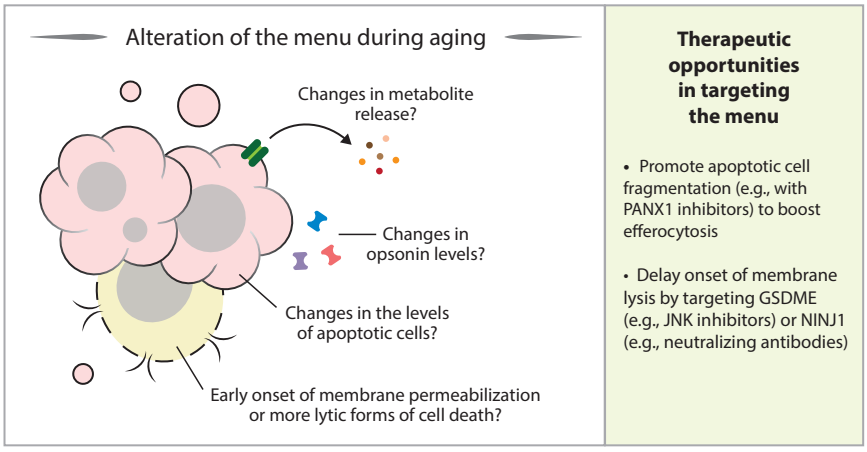

Figure 3

Alteration of the menu during aging and therapeutic opportunities in targeting the menu to boost efferocytosis. (*Left*) Aging can potentially alter various properties of dying cells and subsequently influence the cell clearance process. This may include changes in the release of metabolites from dying cells or the way in which dying cells are decorated with opsonins. The levels of cell death and cell lysis may also increase during aging. (*Right*) Apoptotic cells can be manipulated therapeutically to boost efferocytosis by promoting cell fragmentation (e.g., with PANX1 inhibitors) or delaying membrane permeabilization (e.g., NINJ1 neutralizing antibodies). Abbreviations: GSDME, gasdermin E; JNK, Jun N-terminal kinase; NINJ1, ninjurin-1; PANX1, pannexin 1.

Besides whole apoptotic cells, soluble factors released by apoptotic cells could also be developed as novel therapeutics. As noted above, a variety of metabolites such as spermidine, GMP, inosine monophosphate (IMP), fructose-1,6-bisphosphate (FBP), dihydroxyacetone phosphate (DHAP), and uridine diphosphate (UDP)-glucose are released by dying cells during early stages of apoptosis (18). Remarkably, a combination of these metabolites (e.g., spermidine, GMP, and IMP) can be used in isolation to reduce inflammation in a mouse model of serum-induced arthritis (18). Notably, the natural polyamine spermidine has been investigated extensively in relation to aging, and the levels of spermidine have been reported to decline in aged model organisms (e.g., *Saccharomyces cerevisiae*, *Drosophila*, mouse, rat) as well as in elderly humans (125). Most strikingly, dietary spermidine supplementation can prolong the life span of model organisms and humans, perhaps via its effect on multiple hallmarks of aging (125). These studies suggest that metabolites released by apoptotic cells could be explored as cell-free therapeutics for aging-related diseases. Furthermore, EVs generated by apoptotic cells have also been developed as therapeutics. For example, large EVs such as ApoBDs generated from mature osteoclasts carry membranous receptor activator of nuclear factor kappa-B (RANK) on the EV surface that can activate RANK ligand (RANKL) reverse signaling in pre-osteoblasts to promote osteogenic differentiation (126). Although the link between inflammaging and osteoporosis is still emerging (127), it is worth considering whether ApoBDs carrying distinct factors such as RANKL could be developed as cell-free therapeutics to help restore defects in osteoblast differentiation in the aging skeleton. More recently, small exosome-like EVs generated from apoptotic mesenchymal stem cells (MSC-ApoEVs) have also been explored extensively as a therapeutic to treat many pathological conditions, including myocardial infarction, allergic lung inflammation, and osteopenia (128). Collectively, these studies highlight how cell-free therapies harnessing factors released by apoptotic cells can be used as treatments for a variety of inflammatory diseases associated with aging and even prolong the life span.

5. CONCLUSION

Twelve hallmarks of aging have been described, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis (69). As described in this review, a number of these hallmarks such as mitochondrial dysfunction, cellular senescence, altered intercellular communication, and chronic inflammation can directly affect the efficiency of efferocytosis. Conversely, impaired efferocytosis can fuel the chronic inflammation that occurs during aging. Similar to proteostasis and macroautophagy, efferocytosis is another important mechanism to safeguard the organism from harmful endogenous factors that reside benignly within healthy cells. The emerging link between efferocytosis and inflammaging and the multidimensional relationship between efferocytosis and hallmarks of aging remain to be fully defined. Nevertheless, it is clear that an optimal level of efferocytosis is important for healthy aging, and a better understanding of how aging may impair the efferocytic process will lead to new therapeutic opportunities to restore or boost efferocytosis to limit inflammaging.

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