REVIEW

Role of Efferocytosis in Health and Diseases

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Abstract

Efferocytosis is the process of removal of apoptotic cells through phagocytosis by the specialized cells known as efferocytes. Clearance of dead cells also plays an important role in the defensive system of organisms as efferocytosis maintains homeostasis and repairing of tissues and organs. In this process, the dying cell releases signals for identification and engulfment which is further processed by macrophages. Efferocytosis prevents the secondary necrosis and release of pro-inflammatory cellular contents. This clearance process involves interplay of signaling molecules, receptors, and other mediators that ensures prompt recognitions and removal of dying cells. Dysregulation of efferocytosis has been implicated in various pathological conditions, including autoimmune diseases, chronic inflammation, and atherosclerosis. This review focuses on some common autoimmune diseases, cardiovascular diseases, respiratory disorders, and neurodegenerative disorders due to impaired efferocytosis. To describe the pathophysiology of efferocytosis in diseases more extensive studies are required.

Key words: Atherosclerosis, Autoimmune disorder, LC3-associated phagocytosis

Introduction

Programmed cell death (PCD) usually takes place during growth and aging and as a homeostatic mechanism by removing unwanted cells. PCD is required to protect the normal cells (Liu et al., 2022). PCD acts as a defensive process in immune responses or when cells are affected by disease or toxins. Efferocytosis is a process in which dying cells are removed by phagocytic cells, especially by macrophages (Razi et al., 2023). It can also refer to the "burial of dead cells". Efferocytosis resembles phagocytosis but a distinct process, with major difference is that efferocytosis inhibits proinflammatory cytokines and produces anti-inflammatory cytokines that make it immunologically silent state, while proinflammatory cytokines are produced in phagocytosis (Gheibi Hayat et al., 2019).

In the developing vertebrate nervous system, after the formation of neurons more than half of the cells and in thymus, 95% of T-lymphocytes undergo apoptosis. In an adult human, nearly 0.4% of the approximated 37.2 trillion cells turnover every day (Doran et al., 2020) and in the intestine and bone marrow billions of cells undergo apoptosis ever hour (Gheibi Hayat et al., 2019). In humans and mice, it is studied that quick clearance of dying cells is essential to maintain cell population in tissues and immune tolerance. Impaired clearance of apoptotic cells (ACs) is linked with several inflammatory diseases (Ge et al., 2022). Clearance of ACs by efferocytosis is usually non-inflammatory and doesn't induce autoimmunity (Tajbakhsh et al., 2022).

Professional phagocytes (macrophages and dendritic cells) with the primary function of efferocytosis and nearby nonprofessional phagocytes (fibroblasts, endothelial and epithelial cells) without the primary function of efferocytosis perform the removal of dead cells by efferocytosis (Boada-Romero et al., 2020; Yoshimura et al., 2020). Dendritic cells (professional phagocytes) engulf unwanted cells and pass antigens from these apoptotic cells to T cells by a process called cross-presentation. Apoptotic cells discharge "find-me" signals to trigger the phagocytes to identify the "eat-me" signal on the dying cell followed by extensive cytoskeletal rearrangement in the phagocytes to engulf dead cells leading to release of anti-inflammatory mediators after rocessing the engulfed cells (Boada-Romero et al., 2020). In some conditions cell death is not instantly followed by corpse disposal as it is reported in the shedding epithelial cells from the

villi tips into the lumen of the intestine, However, in most situations, efferocytosis clears the apoptotic bodies soon after their death (Boada-Romero et al., 2020).

Removal of dead cells by efferocytosis

Cell death and their effective clearing is the basic process to maintain homeostasis in multicellular organisms. In human body, 200–300 billion cells are replaced daily, mostly by means of caspase-dependent pathway. It is also essential for growth and development as billions of cells are removed during the embryogenesis and organogenesis (Boada-Romero et al., 2020; Fuchs & Steller, 2011; Mehrotra & Ravichandran, 2022; Suzanne & Steller, 2013).

Cell death, during different pathological processes needs to be managed as, dying cells release intercellular substances in the microenvironment which leads to activation of inflammatory pathways affecting the neighbouring cells. (Boada-Romero et al., 2020). Phagocytosis is a multistep process mediated by the soluble mediators secreted by dying cells to attract phagocytes, which are responsible for clearing the dead cells from the tissues by efferocytosis (Krysko & Vandenabeele, 2010). One of the advantages of efferocytosis is that it rapidly removes the apoptotic cells and avoids the onset of secondary necrosis, protecting the rest of the tissue from any inflammation. Secondly, phagocytosed dying cell turns to a "pro-resolution phenotype" through boosting the release of anti-inflammatory mediators and stimulating the "pro-repair transcriptional" schemes (Mehrotra & Ravichandran, 2022).

Identification of dead cells

Cell reduction before the death is associated with a non-inflammatory system for clearance of the cells, whereas cell death is coupled with an inflammatory reaction. These are related to the mechanisms of PCD and necrosis. (Galluzzi et al., 2018), that significantly impact the natural cost of cell death. Programmed and non-programmed cell corpses show and discharge molecular signals to phagocytes and express successive phagocytic and immune reactions (Boada-Romero et al., 2020).

"Find-me" signals

The type of "silent" cell death is apoptosis. The dead cell is digested by activated inflammatory mediators and their substrates, enclosed into apoptotic cells, and then eliminated and recycled by adjacent phagocytes, avoiding the discharge of inflammatory cellular components and inflammation. During the process of death, the cell discharges some soluble messages to the surrounding like Lysophosphatidylcholine (LPC) and sphingosine 1-phosphate (S1P); nucleotides, including ATP and UTP16 (Elliott et al., 2009); and chemokines, such as CX3CL1 also named as fractalkine (Truman et al., 2008) to activate the macrophages and enhance their scavenging ability (Figure 1). LPC and S1P are one of the apoptotic "find-me" messages. During cell death, caspase-3 splits and engages calcium-independent phospholipase-A2 to derive LPC from phosphatidylcholine (Lauber et al., 2003).

These factors act as phagocytes attractive signal to attract them to the apoptotic cells and prepare them to phagocytose the dying cell by modifying their cytoplasm by enhancing the phagocytosis receptors and the digestion apparatus expressions

(Medina et al., 2020). Sphingosine kinases (SPK1 and SPK2), phosphorylates the membrane lipid sphingosine to yield S1P, are upregulated in some dead cells (Gude et al., 2008; Weigert et al., 2006).

Signals from non-apoptotic cells

Non-apoptotic cell death like necrosis, involves cell rupture releasing the cell contents like genomic and mitochondrial DNA, nuclear proteins with high-mobility group (HMG) protein B, histones, cytoplasmic proteins, Interleukin cytokines (IL-1, IL-33, IL-36), and other relatively small substances such as ATP, UTP, uric acid crystals and damage-associated molecular patterns (DAMPs), which acts as inflammatory signals and trigger an inflammatory response in the neighbouring cells. Pathogens infested cells can release pathogen-associated molecular patterns (PAMPs), acquired from microbes in contrast to DAMPs, which are intracellular that triggers pattern identification receptors on or within the phagocytic cells (Boada-Romero et al., 2020). DAMPs lead to an inflammatory response by acting as macrophage chemoattractant. (Scaffidi et al., 2002; Shi et al., 2003).

"Eat-me" signals

Along with to generation of "find-me" responses, dead cells present "eat-me" surface signals, like phosphatidylserine, which helps the phagocytes to distinguish dead cells from healthy neighboring cells (Boada-Romero et al., 2020). LPC on the cell membrane of the ACs can attach to Immunoglobulin M (IgM), which then holds to fragment crystallizable (Fc) receptors on phagocytic cells, such as macrophages (Kim et al., 2002).

The capability of phagocytes to identify apoptotic bodies depends upon the contact of "eat-me" signals on ACs surface while "do not eat-me" signals are exposed on the surface of living cells (Kelley & Ravichandran, 2021). In the absence of don't-eat-me signals, proteins residing in the lumen of the ER, like calreticulin, can be revealed at the surface of ACs and behave as an "eat-me signal". Calreticulin is detected through the LDL receptor-related protein 1 which is also known as CD91 by phagocytes, works in cooperation with C1q, a complement element and mannose-binding lectin (MBL) (Gardai et al., 2005; Ogden et al., 2001).

"Don't-eat-me" signals

When ACs exhibit eat-me signals, healthy cells exhibit the don't-eat-me signals to avoid being engulfed. Two cell surface proteins CD47 and CD24 are "don't-eat-me signals, which are identified by the receptors signal-regulatory protein-α (SIRPα) and sialic acid binding Ig-like lectin 10 (SIGLEC10) on macrophages (Barkal et al., 2018; Oldenborg et al., 2000). Interaction of cell surface CD31 of healthy cells and macrophages is attributed to evade phagocytosis (Figure 1) (Brown et al., 2002). Furthermore, in healthy cells, Class I MHC compounds stimulate the LILRB1 (leukocyte immunoglobulin like receptor B1) inhibitory receptor, which restricts the phagocytosis and prevents the response to inflammatory messengers (Barkal et al., 2018).

Mechanisms of phagocytosis of dead cells

Efferocytosis is a synchronized process that involves the timely uptake of ACs by macrophages (Boada-Romero et al., 2020).

Following the uptake of dying cell, it progresses through a lysosomal system to be completely digested (Richards & Endres, 2014). Cell eating starts when "eat-me signals" bind with the phagocytic cell surface receptor followed by an actin-mediated uptake of the AC into a phagosome. Phagosome maturation is regulated by the activity of Phosphatidylinositol 3-kinase (VPS34), phosphatidylinositol-3-phosphate (PI3P), and RAB proteins (Flannagan et al., 2012; Rubino et al., 2000). The blending of the late phagosome with the lysosomes is regulated by syntaxin and vesicle-associated membrane protein 7 (VAMP7) which permits lysosomal components to digest the phagocytic load. Phagocyte maturation is attained by rising acidity in the lumen, regulated by V-ATPase (Kissing et al., 2015).

Microtubule-associated protein (1A/1B light chain 3) and LC3-associated phagocytosis (LAP) is considered to be active on the phagosome wall which induces formation of a PI3K complex (phosphatidylinositol 3-kinase), comprising of Rubicon, UVRAG, Beclin 1, VPS34, and VPS15, leading to establishment of the LC3 ligation machinery (Heckmann et al., 2019; Martinez et al., 2016; Nakamura & Yoshimori, 2017). This causes the effective removal of the dead cells. If LAP is impaired, it obstructs the phagosome maturation and lysosomal fusion with the phagocytosed dead cell leading to inflammatory immune responses (Boada-Romero et al., 2020).

Role of efferocytosis in host defense

Macrophages and neutrophils are the first line of defense against foreign microbes by phagocytosis of dying as well as bacterial cells by efferocytosis. Following the death of cells phagocytes are developed with huge bacterial masses, with potential risk of leaking out of the tissues (Hosseini et al., 2016). Phagocytic activity of macrophages to remove the dead cells removal of bacteria and protects the cells and tissues from onset of secondary necrosis (Doran et al., 2020).
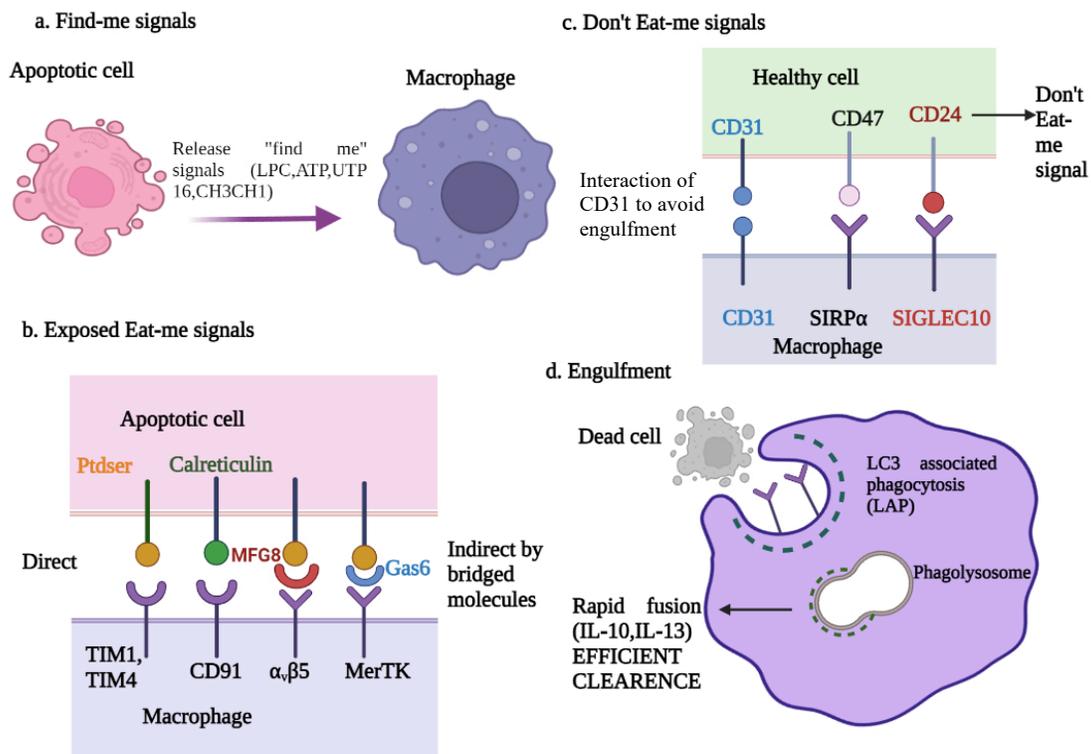

Figure 1 Generalized mechanism of efferocytosis; a) Apoptotic cells release some "find-me" signals such as LPC, UTP16, ATP, and CH3CH1 to activate macrophage and enhance their scavenger ability. b) Apoptotic cells show "eat-me" signals on the cell surface which separates dead cells from healthy neighboring cells. Phosphatidylserine (Ptdser) is an effective eat-me signal and macrophages have Ptdser recognition sites on their surface. Due to Ptdser sites receptors are attached directly while some molecules need bridges. c) To prevent engulfment by macrophage, the healthy cell shows some "don't eat-me" signals such as CD24, and CD47. Also, the interaction of CD31 on healthy cells and macrophages avoids engulfment. d) Receptors to recognize dead cells are present on the macrophage surface, engulfment occurs by maturation of the phagosomes and splitting of phagolysosomal components. Microtubule-associated protein (1A/1B light chain 3) and LC3-associated phagocytosis (LAP) is characterized by the activity of LC3 to the wall of the phagosome. Rapid fusion leads to efficient clearance while impaired engulfment cause inflammatory immune responses. Source: Created with BioRender.com

In humans, *Mycobacterium tuberculosis* is responsible for a bacterial disease called pulmonary tuberculosis (TB). *M. tuberculosis*-containing efferocytic phagosomes effectively integrate with lysosomes, leading to the death of bacteria both *in vitro* and *vivo* conditions. When *M. tuberculosis* is up taken by macrophages, bacteria persist in phagosomes due to *M. tuberculosis* induced inhibition of lysosome–phagosome combination. Comparatively, low-virulent strains of *M. tuberculosis* frequently stimulate the programmed cell death. When macrophages uptake the apoptotic cells, lysosomes within the macrophages fuse with the phagosomes containing the apoptotic cells or bacterial cell. This fusion facilitates the breakdown of the ingested material, including the apoptotic cells (Martin et al., 2012). Potent *M. tuberculosis* strains induce

necrosis, manipulating the efferocytosis that contributes to the pathogenicity of M. tuberculosis (Moraco & Kornfeld, 2014).

In case of leishmaniasis caused by the parasite *Leishmania major*, the infecting bite of sandfly attracts neutrophils, which rapidly phagocytose *L. major* metacyclic promastigotes, which is vital for disease initiation. Infection with the parasite triggers the death of neutrophils, that attracts the macrophages and dendritic cells which phagocytose the *L. major* infected neutrophils *in vivo* (Ribeiro-Gomes et al., 2012; van Zandbergen et al., 2004). Macrophage engulfment of infested apoptotic cells regulates the conversion of promastigotes to amastigotes, which can prevent phagolysosomal degradation of parasite (Martin et al., 2014).

Some pathogens have evolved mechanisms to hijack the efferocytotic system, exploiting the process of apoptotic cell clearance to facilitate their own survival and replication within macrophages. Vaccinia viruses use this kind of "apoptotic mimicry" by expressing phosphatidylserine (PtdSer) on the surface of infected cells. and mimics the appearance of apoptotic cells, which are more readily engulfed by macrophages. Once inside the phagocytes, the virus can evade immune surveillance and exploit the cellular machinery for replication and spread. (Mercer & Helenius, 2008). In certain conditions, bacteria can prevent efferocytosis mediated mortality by inhibiting infected apoptotic bodies from being swallowed by the macrophages such as endocytosis of methicillin-resistant *Staphylococcus aureus* by neutrophils, which causes CD47 a don't-eat-me signal to be expressed (Greenlee-Wacker et al., 2014). Consequently, deceased infectious neutrophils are not swallowed by macrophages, and the necrosis of neutrophils allows existing bacterial escape.

Efferocytosis is a multi-step homeostatic process that needs the maintenance of various signals, receptors, and phagocytes. Impaired efferocytosis results in multisystem diseases including diabetes, heart diseases like heart attack, atherosclerosis, lung disease, neurodegenerative disorders, and cancer (Cabrera & Makino, 2022).

Efferocytosis Linked with Autoimmunity

Improperly ingested ACs leads to secondary necrosis in which cellular components released due to plasma membrane rupture leads to autoimmunity and constant inflammation (Fernandez-Boyanapalli et al., 2010; Peng & Elkon, 2011). At the beginning of apoptosis, the ACs are identified and ingested by macrophages because of phosphatidylserine (PS) dependent pathway. Genetic proof from mice model studies has confirmed that unsuccessful or improper efferocytosis leads dysfunctions of the immune system (Mũoz et al., 2010).

DAMPs excite the inflammatory and immunogenic responses, which activate an autoimmune reaction. Macrophages identify PtdSer exposure on apoptotic bodies, prompt efferocytosis, and produce anti-inflammatory responses in normal circumstances. PtdSer exposure in ACs leads to improper efferocytosis due to deficiency of Xkr8-scramblase that cause exposure delay. An error in the PtdSer recognition by receptors Tim4, MFG-E8, and TAM receptor kinases leads to impaired efferocytosis (Figure 2) (Kawano & Nagata, 2018).

Impaired efferocytosis has been reported to directly associated with various autoimmune and inflammatory issues like SLE (Systemic Lupus Erythematous), rheumatoid arthritis (RA), type I diabetes, and multiple sclerosis (MS) (Herrmann et al., 1998; Yang et al., 2019).

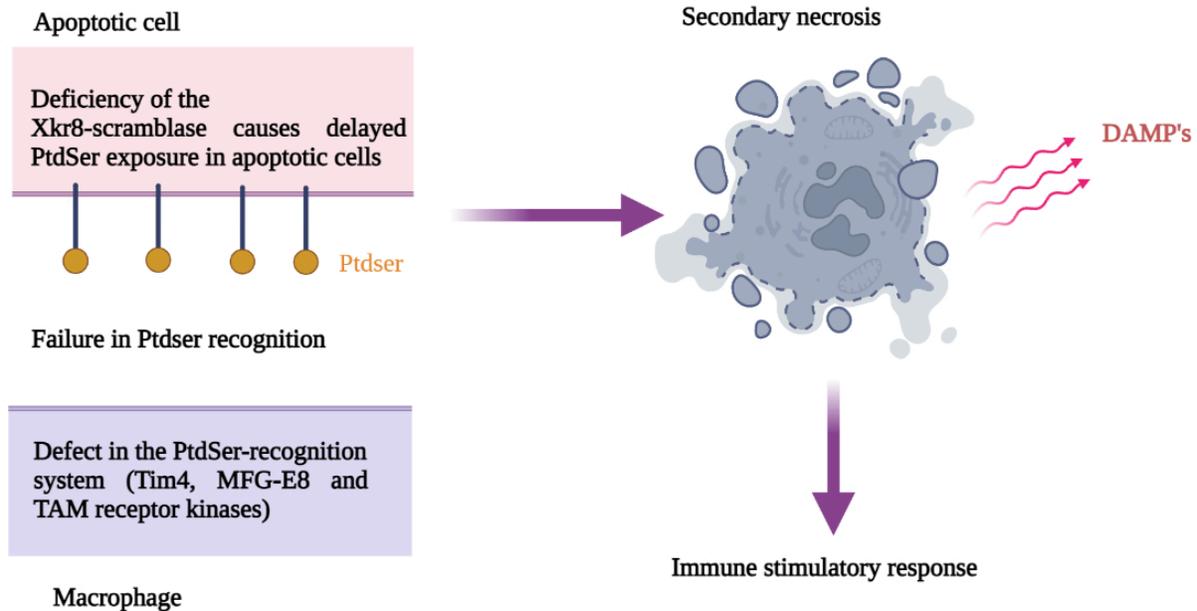## Aberrant efferocytosis in health and diseases:

Figure 2 Efferocytosis and Autoimmunity; If there is improper engulfment of apoptotic cells, they undergo secondary necrosis causing autoimmunity and constant inflammation. Source: Created by BioRender.com

Systemic Lupus Erythematous

One of the autoimmune disorders linked with improper efferocytosis is Systemic Lupus Erythematous (SLE), a chronic autoimmune disease in which the immune system attacks

healthy tissues, leading to inflammation, tissue damage, and a wide range of symptoms (Cancro et al., 2009). SLE mainly affects women between the ages of adolescence and menopause (Kaul et al., 2016). SLE patients express autoantibodies against

cellular contents such as anti-nuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) or anti-single-stranded DNA (ssDNA) antibodies and antiphospholipid antibodies. It can be induced by both internal and external factors (Mũoz et al., 2010).

At the beginning of cell death, C1 combines with dying cells via the IgM-dependent method and through appearance of LPC stimulus on them for IgM-adhesion (Kim et al., 2002). Further, it has been demonstrated that humans lacking the C1q gene are at risk of SLE onset. In mice with C1q absence like MRL/Mp strain, ACs excite the growth of SLE-like glomerulonephritis (Potter et al., 2003). Several mutations in ATG5 and ATG7 genes (Clarke et al., 2015; Zhou et al., 2011), related to autophagy and LAP, have been reported as susceptibility indicators for SLE by genomic analysis (Florey et al., 2011; Henault et al., 2012; Mizushima et al., 2002).

Along with the various organs that may be impaired by SLE, cardiac impairment might be very severe. Particularly, QT interval extension, which can be triggered by cardiomyocyte programmed cell death, chronic inflammation which may raise the risk of cardiovascular disorders (Zhang et al., 2011; Lazzerini et al., 2013; 2016; Schillaci et al., 2006).

Type 1 Diabetes:

Type 1 Diabetes is a T cell-mediated disorder related to autoimmunity due to insufficiency of insulin and hyperglycemia. Ineffective removal of apoptotic cells of the pancreas may lead to secondary necrosis and inflammation (Heimberg et al., 2001).

An imperfect removal of ACs has been found associated with immunogenic responses, dendritic cell maturation, and chronic inflammation (O'Brien et al., 2006). A general characteristic among patients of Type1 and Type2 Diabetes mellitus both possess improper wound healing. Accumulation of dying cells at wound sites can cause inflammation and slow down the healing of wounds because of impaired efferocytosis (Khanna et al., 2010; Maruyama et al., 2007).

Cardiovascular diseases (CVDs):

CVDs, primarily ischemic heart disease (IHD) and stroke are the main reason for death and severe disability worldwide (Roth et al., 2020). Mutation in chromosome 9p21 determines the risk of cardiovascular diseases (Zhang et al., 2022). Impaired efferocytosis leads to cardiovascular diseases like atherosclerosis, coronary artery syndrome and eventually causing myocardial infarction (Figure 3).

Atherosclerosis:

Atherosclerosis is a prolonged inflammatory process in which blood vessels harden and narrow because of the formation of atherosclerotic plaque in impaired vascular walls (Yesiltas et al., 2021).due to the accumulation of low-density lipoprotein (LDL) or bad cholesterol, calcium, fat, and many other substances present in the blood (Zhao et al., 2020). These atherosclerotic plaques can burst and result in thrombosis or heart attack or acute kidney injury which is often fatal (Kojima et al., 2022).
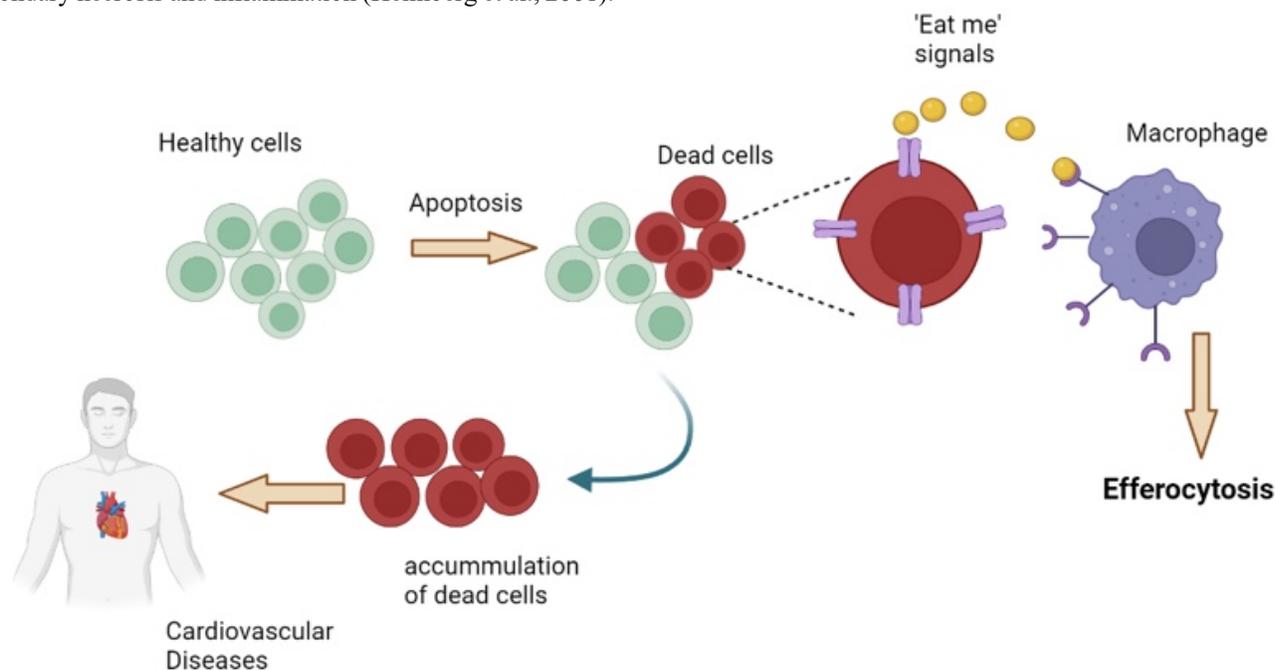

Figure 3: Role efferocytosis in CVDs: In normal conditions, when healthy cells undergo apoptosis, cellular corpses are formed. These cellular corpses release "Eat-me signals" which are detected by macrophages and result in proper efferocytosis. If efferocytosis is impaired, cellular corpses start accumulating thus leading to cardiovascular diseases (CVDs). Source: Created with BioRender.com

Chronic stress is a major risk factor, that damages the arteries endothelial cells and activates macrophages to uptake the oxidized LDL and form the foam cell, which accumulates in the walls of arteries to form plaque (Yao et al., 2019). Accumulation of cholesterol leads to the release of pro-inflammatory cytokine and form cholesterol microcrystals inside the cells and triggers the inflammasome.

Notably, endothelial cells (EC) are in lesion-prone regions, where plaques form with a high turnover rate of endothelial cells due to overwhelmed apoptosis (Hobby et al., 2019). Efferocytosis itself becomes impaired, and/or lesional apoptotic cells become poor substrates for efferocytosis. (Festuccia et al., 2014). For instance in plaque, CD47 expression rate increases, most probably through a TNFα-dependent mechanism

(Yurdagul et al., 2018). During the early stage of atherosclerosis, the level of inflammatory cytokine TNF-α increases which suppresses Milk Fat Globule-EGF and Factor 8 (MFG-E8) protein-coding gene, MER proto-oncogene tyrosine kinase (MerTk) and LDL Receptor Related Protein (LRP1) by triggering Toll-like receptor (TLR) and CD47 expression to start the "don't eat me" signal. Removal of dead cell is impaired due to TNF-α, thus delaying the clearance of apoptotic bodies that triggers an inflammatory response and exacerbating atherosclerosis (Martinet et al., 2011). In these allele carriers' plaques, Calreticulin protein expression decreases, but the area of the necrotic core and the number of apoptotic bodies rise in atherosclerotic plaques. Calreticulin binds to the "eat me" ligand on the surface of ACs, triggering phagocytosis via activating LDLR4 on the surface of phagocytes (Gardai et al., 2005) which inhibits the "eat me" signal and declines the process of phagocytosis of ACs (Cunnington & Keavney, 2011).

Drugs like statins & non-steroidal PPAR γ agonists can be used in this inflammatory atherosclerosis which can prove to be helpful in the clearing the apoptotic cells thus preventing the conditions to become worsening. In atherosclerosis, impaired efferocytosis can lead to secondary necrosis of cellular corpses, the release of inflammatory factors, cholesterol reverse disorder, formation of atherosclerotic plaque, and acute coronary artery syndrome (Zhang et al., 2022).

Respiratory Diseases

Alveolar macrophages act as 1st line of defense against pathogens that are inhaled with air into the lungs (Suzuki et al., 2020). During the inflammation of the lungs, neutrophils are readily drawn to the airways (Zhang et al., 2022). Phagocytosis of invaders leads to PCD of neutrophils that is regulated by several genes and factors including apoptotic genes (Fox et al., 2010) that prevents the secondary necrosis.

Efferocytosis and lung disease:

Alveolar Møs (AMøs) are known to be professional phagocytes of the alveolar space have receptors for apoptotic bodies. Dendritic cells (DCs), macrophages of several tissues, and alveolar møs powerfully express Tyro3, Axl, and Mer receptors (TAM). Inhibiting TAM reduces the uptake of apoptotic cells but does not affect binding. Improper and delayed clearance leads to the build-up of apoptotic cells and activation of inflammatory response in human. Many respiratory disorders are characterized by the accumulation of ACs and prolonged inflammation (McCubbrey & Curtis, 2013) like elastase-induced emphysema in mice (Van Pottelberge et al., 2012).

Chronic obstructive pulmonary disease (COPD):

COPD is characterized by the damage of interstitial matrix, chronic inflammation, and a high rate of programmed cell death of neutrophils and endothelial cells (Lareau et al., 2019). COPD is leads to a decrease in the luminal diameter of the airway due to mucus gland hyperplasia and peri-bronchial fibrosis; damage of pan-alveolar and loss of small airways (Hogg & Timens, 2009). Alveolar destruction and inflammation lead to emphysema (Suzuki et al., 2020). Proteins that are involved in clearance of ACs have altered expression including reduced extracellular pentraxin-3 in small airways (Hodge et al., 2008). In COPD patients, macrophage-mediated clearance of ACs decreased dramatically as compared to the patients who take statins (Noda et al., 2013).

Asthma and Cystic fibrosis:

Asthma is a complex syndrome characterized by irritation, breathlessness, bronchial hyper-responsiveness, and airway inflammation. Cystic fibrosis is a genetic ailment characterized by infection, airway inflammation, and bronchiectasis (Zhang et al., 2022). Impaired efferocytosis has been linked to inflammation and tissue destruction in asthma and cystic fibrosis (Zheng et al., 2021). It has been observed that people suffering from cystic fibrosis had more apoptotic cells in their sputum than those with chronic bronchitis.

Idiopathic Pulmonary Fibrosis (IPF):

The most prevalent type of IPF is a progressive, irreversible, and frequently fatal disease marked by an aberrant fibrotic response affecting large portions of the lungs (Phan et al., 2021). However, the underlying mechanism of this disease is still unknown (Michalski & Schwartz, 2020). TNF-related apoptosis-inducing ligand (TRAIL) is decreased in IPF that has a role in programmed death of neutrophils. TNF-related apoptosis-inducing ligand knockout mice display decreased apoptotic rate of lung neutrophils and more lung collagen in a bleomycin model (McGrath et al., 2012) which proposes that TRAIL can be used as a biomarker as well as potential therapeutics in idiopathic pulmonary fibrosis.

Acute lung injury (ALI)

Acute respiratory distress syndrome (ARDS) along with ALI causes significant death and severe illness in surviving patients (Noone & Reddy, 2021). ALI/ARDS is characterized by the death of alveolar epithelial and endothelial cells, neutrophil alveolitis, and damage of epithelial capillary barriers that result in vascular permeability and edema infiltration (Huppert et al., 2019). Defective efferocytosis influences prognosis in murine models of ALI and ARDS (McCubbrey & Curtis, 2013). Impaired clearance of ACs leads to necrosis and the release of toxic substances including DAMPs, that trigger inflammatory responses by triggering innate and adaptive immune responses (Chen et al., 2021; Zhang et al., 2022).

Neurodegenerative disorders:

During embryonic development, the brain generates far more neurons than are ultimately retained. This "extra" number of nerve cells undergoes PCD or apoptosis which is a crucial step during nervous system development. In the brain, microglia clear the excess of the brain cells and dead aging cells to maintain homeostasis and prevention of secondary necrosis (Hamilton et al., 2020).

Clearance of apoptotic bodies may become defective and lead to neurodegenerative diseases due to aging, inflammation, and specific genetic risk variants causes some pathological conditions (Hall-Roberts et al., 2021; Márquez-Ropero et al., 2020). Efferocytosis is linked to anti-inflammatory responses that remove dead nerve cells. Impaired clearance of these dead neurons results in many diseases of the CNS (Figure 4) (Zhang et al., 2022).

Alzheimer's disease (AD)

AD; a chronic neurodegenerative disorder is characterized by the formation of plaque by deposition of the hyperphosphory-lated protein Tau and Amyloid-β (Aβ) (Shi et al., 2022; Uddin et al., 2020) arranged in a way to form amyloid-like filaments (Shi et al., 2022). Downregulation of MFGE8, (an anti-

inflammatory agent) expression has previously been reported in an animal model of Alzheimer's disease (Fricker et al., 2012). MFGE8 inhibits A1 astrocytes and controls the alternation of microglia M1/M2 which stops nerve cell death and oligodendrocytes by regulating the nuclear factor kappa B (*NF-κB*) and PI3K-AKT. Recombinant MFGE8 can be useful in curing inflammation in AD (Cheyuo et al., 2019), by suppressing MAPK and NF-κB signaling pathways.

Parkinson's disease (PD)

PD; characterized by the accumulation of presynaptic neuronal proteinα-synuclein. Declined dopamine (DA) level in the striatum, is the main pathological change that leads to PD, due to the death of dopamine-releasing nerve cells (dopaminergic neurons) in the midbrain substantia nigra (SN) (Hanss et al., 2021; Naoi et al., 2020; Ugrumov, 2020). Impaired clearance by microglia has been observed in Parkinson's disease and defective phagocytosis by the monocytes has also been reported Parkinson's disease patients.

Neuronal apoptotic bodies are removed by phagocytes through the process of efferocytosis thus autoimmunity and inflammatory responses are suppressed which increases neuronal survival and axonal regeneration (Zhang et al., 2022).
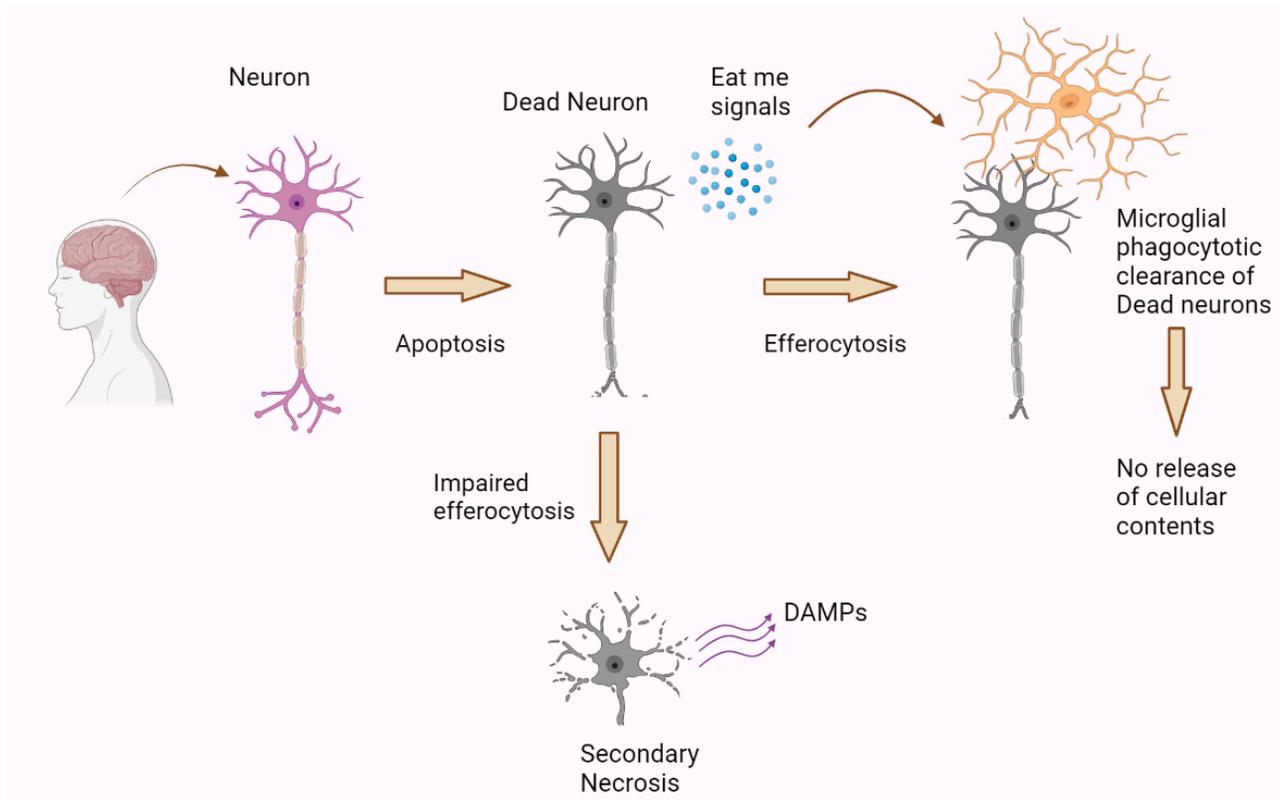

Figure 4: Role of efferocytosis in neurodegenerative disorder: when neurons undergo programmed cell death in a healthy brain, these dead neurons release "Eat me" signals. Professional efferocytes i.e. Microglia detects these signals and clears these corpses thus preventing secondary necrosis. If proper and timely efferocytosis doesn't occur, it will lead to secondary necrosis and release of toxic materials. Source: Created with BioRender.com

Conclusions and future perspectives:

This review discusses the mechanism of efferocytosis and the role of impaired efferocytosis in different diseases including CVDs, neurodegenerative disorders, autoimmune disorders, and respiratory disorders. Efferocytosis is a balanced process by which dead cells are removed by phagocytic cells, perfectly coordinated between "find-me", "eat-me" and "don't eat-me" signals. Removal of infected dead cells is processed by macrophages preventing secondary necrosis of the infected tissues.

Efferocytosis provides therapeutic opportunities in infections caused by pathogens. More extensive studies are required to explain the pathophysiological mechanism of efferocytosis in pathological conditions. Several chemotherapies, radiotherapies, and immunotherapies have been developed but these therapies are not proven to be much effective. Despite of application of these therapies, the mortality rate is still high which indicates their limitations. New effective therapies are needed to resolve this emerging issue.

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