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Review article

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Cell death by phagocytosis

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Abstract

Cells can die as a consequence of being phagocytosed by other cells — a form of cell death that has been called phagotrophy, cell cannibalism, programmed cell removal and primary phagocytosis. However, these are all different manifestations of cell death by phagocytosis (termed 'phagoptosis' for short). The engulfed cells die as a result of cytotoxic oxidants, peptides and degradative enzymes within acidic phagolysosomes. Cell death by phagocytosis was discovered by Metchnikov in the 1880s, but was neglected until recently. It is now known to contribute to developmental cell death in nematodes, *Drosophila* and mammals, and is central to innate and adaptive immunity against pathogens. Cell death by phagocytosis mediates physiological turnover of erythrocytes and other leucocytes, making it the most abundant form of cell death in the mammalian body. Immunity against cancer is also partly mediated by macrophage phagocytosis of cancer cells, but cancer cells can also phagocytose host cells and other cancer cells in order to survive. Recent evidence indicates neurodegeneration and other neuropathologies can be mediated by microglial phagocytosis of stressed neurons. Thus, despite cell death by phagocytosis being poorly recognized, it is one of the oldest, commonest and most important forms of cell death.

Sections

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Introduction

Cell death by phagocytosis — also referred to as 'phagoptosis' — is cell death resulting from a live cell being phagocytosed by another cell, and the engulfed cell dying due to engulfment and digestion within the engulfing cell. Phagocytosis itself is the broader process of engulfment and digestion of any extracellular particle >0.5 μm in size[1]. A defining characteristic and diagnostic test of cell death by phagocytosis is that if phagocytosis is blocked, then cell death is prevented. Three examples of cell death by phagocytosis are the normal turnover of erythrocytes (red blood cells) that are phagocytosed when still alive by macrophages[2,3]; the death of cancer cells due to engulfment by macrophages as a result of antibody opsonization or blockage of signals inhibiting phagocytosis[4]; and an amoeba or neutrophil phagocytosing a bacterium resulting in death of the bacterial cell[5].

Cell death by phagocytosis was discovered by Metchnikoff in the 1880s, when he observed white blood cells phagocytosing pathogens, such as bacteria, resulting in the destruction of the pathogen. His subsequent demonstration that this cell death by phagocytosis contributed to immunity was rewarded with a Nobel Prize in 1908. Thus, cell death by phagocytosis was one of the first forms of cell death to be discovered. In 1892, Metchnikoff also proposed that "phagocytes eat all parts of the organism which have become weak for any reason, while ignoring parts fully capable of living"[6]. We will see below how prescient Metchnikoff's proposal was.

Cell death by phagocytosis may also have been one of the first forms of cell death to evolve — in the sense of being selected for, rather than against. Phagocytosis is widespread in protists (unicellular animals) and metazoa (multicellular animals), but very rare in fungi and plants, and absent in bacteria and archaea[7–9]. Eukaryotes are thought to have originated about two billion years ago, and the last common ancestor of all current eukaryotes is thought to have been a phagotrophic protist, that is, a unicellular animal that lived by phagocytosing other organisms[9–11]. Many protists today (including *Ameoba* and *Paramecium*) survive by phagocytosing other live cells, thus resulting in cell death by phagocytosis[9,10,12]. Cell death by phagocytosis of self cells has also been shown to occur in, for example, *Drosophila*[13–16], nematode worms[17–19] and sea urchins[20].

Despite being one of the earliest discovered forms of cell death, cell death by phagocytosis was poorly recognized until recently for various reasons[21,22]. One reason is that cell death by phagocytosis leaves no cell corpse to indicate that it has occurred and to diagnose the form of death. So, cell death by phagocytosis is normally invisible and leaves no trace — which is one of the benefits to the body — but this is a problem for cell death detectives (see Box 1). Another possible reason is that cell death by phagocytosis has different names in different contexts, including erythrophagocytosis, eryptosis, haemophagocytosis, phagotrophy, antibody-dependent cellular phagocytosis, cell cannibalism, programmed cell removal, primary phagocytosis and engulfment-promoted cell death. However, where these processes cause cell death, they cause cell death by the same mechanism, as outlined below and in Fig. 1. A third possible reason why cell death by phagocytosis is poorly recognized is if this process is conceived of as a clearance process, rather than a form of cell death. However, these views are not mutually exclusive, and both may be useful.

In this Review, I discuss the current understanding of phagoptosis, including the mechanisms involved in phagoptosis, its relationship to other forms of cell death and its biological significance, for instance, in the settings of infection, cancer and ageing.

Relationship of phagoptosis to other forms of cell death

Cell death by phagocytosis — that is, phagocytosis of a live cell, resulting in its death — should be distinguished from phagocytosis of dead or dying cells, for example, phagocytosis of an apoptotic cell (known as efferocytosis), where phagocytosis does not contribute to the death of the cell but, rather, to its clearance, and therefore is sometimes known as 'secondary phagocytosis'[23]. Primary phagocytosis means the same thing as cell death by phagocytosis, namely cell death of a target cell as a result of it being phagocytosed by another cell[24,25]. 'Entotic cell death' is the death of a cell that has invaded into another cell by entosis[21]. Entosis normally occurs in the context of the invasion of tumour cells or epithelial cells into their neighbouring cells, following the detachment of the cell from the extracellular matrix[26]. Entotic cell death and efferocytosis can be distinguished from cell death by phagocytosis, as in the latter case inhibition of phagocytosis prevents cell death.

'Programmed cell removal' refers to phagocytosis of both dying cells and viable cells, and thus includes both efferocytosis and cell death by phagocytosis[27]. The concept is concerned with the removal of the cell, rather than with how the cell dies. Similarly, 'cell cannibalism' refers to cells eating other cells, which may be dead or alive, and engulfed cells may survive within the engulfing cell[28,29]. The cell cannibalism term has been primarily used to refer to cancer cells eating other cancer cells[30], but has also been used to refer to cancer cells eating live, non-transformed, host cells[31]. When the engulfed cell is eaten alive and subsequently dies as a result of this phagocytosis, then this is equivalent to cell death by phagocytosis.

The term 'engulfment-promoted cell death' was originally used by Marín-Teva et al.[32] to refer to the death of Purkinje cells (a class of inhibitory neurons) in the developing cerebellum. The death of these neurons was promoted by microglial engulfment and prevented by eliminating microglia. This might represent cell death by phagocytosis, although the engulfed neurons showed signs of apoptosis, such as caspase activation, promoted by the oxidant production accompanying engulfment[32]. Cell death by phagocytosis of nurse cells by surrounding epithelial cells in *Drosophila* also appears to induce caspase activation in the engulfed cell[13]. Cancer cell phagocytosis of natural killer cells also normally leads to apoptosis of the engulfed cell[33]. Thus, there is limited evidence that cell death by phagocytosis may induce apoptosis of the engulfed cell in some cases. However, this requires further investigation, and, as outlined below, is not the normal means of killing the engulfed cells. Moreover, how widespread this form of cell death is requires further investigation.

During developmental cell death in nematodes, caspase activation can induce apoptotic cell death followed by phagocytosis of the cell corpse by neighbouring cells[19]. Apoptotic cell death is associated with cell surface exposure of phosphatidylserine, which can act as an eat-me signal for phagocytes. However, for some cells in nematodes, caspase activation is not enough to induce apoptotic cell death but is sufficient to induce phosphatidylserine exposure and phagocytosis of the live cell by neighbouring cells, resulting in cell death by phagocytosis[17–19]. Caspase activation and phosphatidylserine exposure can be reversible in many different cell types; for example, inflamed phagocytes can induce sub-toxic caspase activation and reversible phosphatidylserine exposure on target cells, inducing cell death by phagocytosis[34]. Thus, markers of apoptosis, such as caspase activation, are not necessarily markers of apoptotic cell death. Furthermore, in some cases, sub-lethal induction of apoptosis pathways may ultimately induce cell death

by phagocytosis, whereas in other cases, cell death by phagocytosis may induce apoptosis of the engulfed cell. However, the distinction between cell death by phagocytosis and apoptotic cell death remains clear: inhibiting phagocytosis will prevent the former but not the latter.

Mechanisms of phagoptosis

How phagocytes recognize targets

Phagocytes recognize targets for phagocytosis using specialized receptors on their surface. These receptors recognize either non-self molecular patterns, eat-me signals or opsonins on the target cell, and then induce engulfment of those targets. Phagocytosis of pathogens by animal cells mainly occurs via scavenger receptors, which are a diverse family of about 26 receptors that recognize a broad range of polyanionic ligands that can be found on pathogens or on host cells[5,35]. Phagocytic receptors that directly recognize the eat-me signal phosphatidylserine on target cells may include T cell immunoglobulin and mucin domain 1 (TIM1), TIM4, stabilin 2, brain-specific angiogenesis inhibitor 1 (BAI1) and triggering receptor expressed on myeloid cells 2 (TREM2)[5]. Note, however, that the evidence that the binding of these receptors to phosphatidylserine mediates phagocytosis of cells is relatively weak, except in the case of TIM4.

Phagocytes can also recognize targets indirectly via opsonins[5,36]. Opsonins are normally soluble, extracellular proteins that when bound to targets induce phagocytes to phagocytose those targets by acting as bridging proteins between the target and the specific phagocytic receptors that they activate. Classical opsonins are IgG and IgM antibodies (which activate Fc receptors on phagocytes) and various complement factors, including C1q (which can activate low-density lipoprotein receptor-related protein 1 (LRP1) or multiple EGF-like domains 10 (MEGF10)), C3b (which activates complement receptor 1 (CR1)) and the C3b derivative iC3b, which activates CR3 (also known as $\alpha_M\beta_2$ integrin)[36,37]. Antibody binding to cell surface-expressed antigens can enable the phagocytosis of pathogenic, cancerous, infected or senescent cells. Non-classical opsonins include milk fat globule-EGF factor 8 (MFGE8), which binds phosphatidylserine on target cells and activates the vitronectin receptors ($\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins) on phagocytes, and growth arrest specific 6 (GAS6) and protein S, which both bind to phosphatidylserine on target cells and activate MER tyrosine kinase (MERTK) receptors on phagocytes. Other non-classical opsonins bind sugar residues normally hidden by terminal sialic acid residues. These include calreticulin, which binds exposed sugars and then activates LRP1; galectin 3 (GAL3), which binds exposed galactose residues and then activates MERTK; and mannose-binding lectin (MBL), which binds exposed mannose and other sugar residues and then activates LRP1, although MBL also activates complement[36,37]. There are currently about 30 known opsonins that bind a wide range of molecules on target cells[36].

Relatively little is known about how phagocytic protozoa recognize and ingest their prey, but there is evidence for the use of opsonins and lectin receptors that recognize mannose residues on the prey surface, similar to what is seen for mammalian phagocytes[38]. However, the amoeba *Dictyostelium discoideum* uses the G-protein-coupled receptor FAR1, which binds sugars on bacterial LPS to enable both chemotaxis towards and phagocytosis of bacteria[9].

Phagocytes also have inhibitory phagocytic receptors that recognize don't-eat-me signals on target cells, preventing phagocytosis of such targets. For example, the don't-eat-me signal CD47 on target cells is recognized by signal regulatory protein-α (SIRPα) on phagocytes to inhibit phagocytosis[39]. Sialic acid residues on the surface of target cells also inhibit phagocytosis via inhibitory sialic acid-binding immunoglobulin-type lectin (SIGLEC) receptors on phagocytes, so removal of these sialic acid residues (termed desialylation) can activate phagocytosis of these desialylated target cells[36]. Some extracellular proteins (termed phagocyte suppressants) bind phagocytes and supress phagocytosis. Examples include vitronectin, histone 3, oxidized low-density lipoprotein (oxLDL), hyaluronic acid, surfactant protein A and surfactant protein D, which inhibit phagocytes either by activating inhibitory receptors or by blocking stimulatory receptors[36].

Phagocytes normally have a limited capacity to phagocytose live cells, but can become licensed to kill by inflammation. Inflammation upregulates the expression of multiple components of the phagocytic machinery, leading to increased expression and release of opsonins and increased expression and activation of phagocytic receptors[1]. The integrin phagocytic receptors (CR3 and vitronectin receptors) require inside-out activation by inflammatory signalling[1]. CR3, MERTK and TREM2 may be outside-in activated by desialylation of the receptors[36]. Desialylation of phagocytes may also activate phagocytosis by removing inhibitory SIGLEC signalling[36]. Inflammatory activation of macrophages via particular pathways can result in their phagocytosis of

Box 1

Imaging cell death by phagocytosis

Imaging phagoptosis can be difficult because it requires continuous or serial imaging of the same cells over time; phagoptosis is a relatively rare event, but then occurs quickly; and detecting whether the cell being phagocytosed is alive or capable of living, if phagocytosis was prevented, can be difficult. Nevertheless, various methods have been devised. Video imaging of activated microglia and neurons in culture showed microglia engulfing intact neurons with uncondensed nuclei, and when phagocytosis was prevented the neurons remained viable[24]. In vivo, retinal microglia were observed to engulf the somata of intact photoreceptors (rod cells), lacking any markers of apoptosis or DNA damage, and when phagocytosis was prevented the photoreceptors remained viable[120]. The viability of lymphoblasts during phagocytosis was monitored by staining the cells with JC-1, which stains the mitochondria red if they are polarized and green when depolarized — addition of anti-CD47 antibodies induced macrophages to engulf polarized (viable) lymphoblasts, which then depolarized after engulfment, indicating phagocytosis of viable cells[102]. Similarly, microglial cell engulfment of viable oligodendrocyte precursor cells was confirmed in brain slices using the dye DilC1(5) to monitor mitochondrial polarization in the oligodendrocyte precursor cells, and in vivo the oligodendrocyte precursor cells were caspase 3-negative when engulfed by microglia[73]. Imaging of *Drosophila* testes, using LysoTracker to monitor lysosomal acidification, and DAPI and TUNEL to monitor DNA condensation and damage, showed that cyst cells phagocytosed sperm progenitors prior to any DNA damage, and when phagocytosis was prevented the progenitors remained viable[16,142]. Thus, imaging phagoptosis is possible; however, doing this in vivo remains challenging.

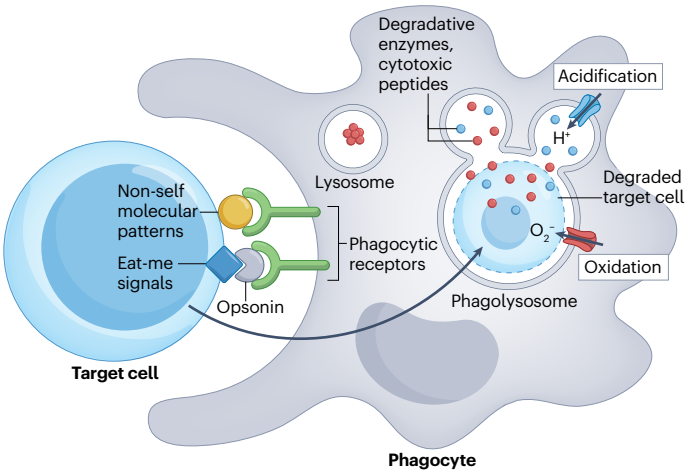

Fig. 1 | Cell death by phagocytosis. Cells targeted for cell death by phagocytosis express eat-me signals or non-self molecular patterns on their cell surface, which bind opsonins and/or phagocytic receptors on the phagocyte. These interactions induce phagocytosis of the target cell into a phagosome, where the NADPH oxidase produces oxidants that permeabilize the target cell membrane. The phagosome subsequently fuses with lysosomes or other vesicles to form a phagolysosome containing cytotoxic peptides and degradative enzymes, which are activated by acidification. Cells that specialize in phagocytosis are known as phagocytes. Target cells are the cells engulfed by phagocytes, and can be pathogens, prey or host cells. Pathogenic or prey cells are recognized as such by phagocytes via receptors or opsonins binding to non-self molecular patterns, often glycans. By contrast, host cells can be phagocytosed following their exposure of eat-me signals.

live B cells, T cells and myeloid cells (known as haemophagocytosis), and this is characteristic of haemophagocytic lymphohistiocytosis and other inflammatory conditions[40].

How target cells induce or deter phagocytosis

Cells can induce phagocytes to phagocytose them by releasing find-me signals, exposing eat-me signals, binding opsonins and removing don't-eat-me signals. Find-me signals are chemotactic molecules released by target cells to chemoattract phagocytes to the target cell prior to phagocytosis. They include the complement components C3a and C5a, formyl peptides, CX_3C-chemokine ligand 1 (CX_3CL1; also known as fractalkine), CXC-chemokine ligand 8 (CXCL8; also known as IL-8), sphingosine-1-phosphate and the nucleotides ATP, ADP, UTP and UDP — these mediators collectively activate various chemotactic receptors on phagocytes[36].

As noted above, phosphatidylserine acts as an eat-me signal when exposed on the cell surface and its exposure used to be thought of as a marker of apoptosis or necrosis. However, it is now clear that many, if not most, live cells can reversibly expose phosphatidylserine on their cell surface due to transient activation of scramblases or inhibition of flippases by calcium or oxidants, followed by phosphatidylserine re-uptake by flippases[41–44]. In some cases, this phosphatidylserine exposure is sufficient to drive phagocytosis of live cells[45], but in other cases, other signals, opsonins or receptors may be required.

Apoptotic or viable cells can release calreticulin onto the cell surface to act as eat-me signals[46,47]. Calreticulin is mainly found in the endoplasmic reticulum, but can be released extracellularly, for example during inflammation or endoplasmic reticulum stress[47,48]. The mechanisms of calreticulin release are not entirely clear, but its release can occur as part of the 'integrated stress response', resulting in endoplasmic reticulum dysfunction and translocation of calreticulin from the endoplasmic reticulum to the Golgi to the extracellular space[48]. In response to inflammation, macrophages and microglia can release calreticulin, which then opsonizes cells by binding desialylated glycoproteins and glycolipids on the target cell and LRP1 on phagocytes[47]. Thus, the release of calreticulin onto the cell surface can be regarded as 'self-opsonization'; as can the release of various other putative eat-me signals, such as annexin A1, histones, DNA and pentraxin 3 (ref. 36).

Terminal sialic acid residues on cell surface glycoproteins and glycolipids act as a don't-eat-me signal, but removal of these and subsequent residues successively exposes galactose, *N*-acetylglucosamine or mannose residues, which can act as eat-me signals by directly activating CR3, macrophage galactose lectin (MGL), the Ashwell receptor (scavenger receptor E4) or the mannose receptor (scavenger receptor E3), or by binding the opsonins calreticulin, C1q, GAL3, MBL or ficolin 2 (refs. 35,36). Thus, exposed sugar residues on the cell surface act as key regulators of whether a cell is phagocytosed or not, and this exposure is affected by inflammation and cellular ageing[36,49].

Cells express or expose don't-eat-me signals to inhibit phagocytosis of themselves by phagocytes. Don't-eat-me signals include CD47, CD24, MHC class I and sialic acid residues on the cell surface[36,39,50]. There are various other proteins that when bound to cells inhibit phagocytosis of those cells, including annexin A5 (ref. 51), sRAGE (soluble receptor for advanced glycation end products)[52], HMGB1 (high mobility group box 1)[53], sMERTK (soluble MER tyrosine kinase)[54], PAI-1 (plasminogen activator inhibitor-1)[55] and PTX3 (pentraxin 3)[56]. Thus, these proteins act as 'negative opsonins', as they are normally soluble proteins in the extracellular space, but when bound to target cells they block phagocytosis of the target cell by phagocytes[36]. Release of adenosine can also act as a soluble don't-eat-me signal, inhibiting nearby phagocytes[57], whereas release of UDP can act as a soluble eat-me signal[58].

Ultimately, whether a cell is phagocytosed or not by a phagocyte will be determined by the mix of eat-me signals, opsonins and don't-eat-me signals that are present on its cell surface, and also on the particular phagocytic receptors expressed by the phagocyte (Fig. 2). Exposure of phosphatidylserine alone may be sufficient to induce phagocytosis of live cells for some cell types and conditions[45], but not others[59], and exposure of calreticulin alone can be sufficient for phagocytosis, at least in some cells and conditions[46,47]. However, phagocytosis of a phosphatidylserine-exposed cell generally inhibits the presentation of antigens derived from that cell; by contrast, phagocytosis of a calreticulin-exposed cell can lead to presentation of antigens derived from that cell[48].

How does the engulfed cell die?

In terms of how cell death occurs following phagocytosis, there are some mechanistic differences depending on the type of phagocyte or target cell involved. However, there are surprising similarities, for example, in how an amoeba kills engulfed bacteria and how a mammalian macrophage does the same thing[5,9,60]. In most cases, death of the engulfed cell results from at least one of the following mechanisms: oxidants generated from the NADPH oxidase that permeabilize the membrane; cytotoxic peptides delivered from phagocyte vesicles; or enzymes delivered from phagocyte lysosomes that degrade the engulfed cell, in a process enabled by acidification of the phagolysosome[5,9,60,61]. The NADPH oxidase is activated within seconds of phagocytosis, and causes

permeabilization of the engulfed cell membrane within 1–2 min[60]. The NADPH oxidase produces superoxide and derivative oxidants: hydrogen peroxide (if superoxide dismutase is present), peroxynitrite (if nitric oxide is present), the hydroxyl radical (if iron is present) and hypochlorite (if myeloperoxidase is present) — which can all peroxidize membrane lipids, permeabilizing the membrane[62–65]. In some cases, cytotoxic peptides from vesicles or granules may contribute to permeabilizing the membrane[5,66]. Fusion of lysosomes with the phagosome releases proteases, glycohydrolases and lipases that degrade the engulfed cell, but can also permeabilize the cell if the NADPH oxidase has not done so already[60]. Acidification of phagolysosomes to pH 4 — which is driven by the H^+-ATPase and NADPH oxidase — activates digestive enzymes, but may also directly contribute to killing target cells[67] (Fig. 1). However, in some specific cases, apoptosis induced by oxidants from the NADPH oxidase may contribute to target cell demise[32,33].

Having outlined the mechanisms of cell death by phagocytosis above, some of the key biological roles of this form of cell death are outlined below.

Biological roles of phagoptosis

Development and stem cell regulation

Phagocytosis is required for the programmed cell death of various cells during the development of the nematode *Caenorhabditis elegans*[17–19]. During oogenesis in *Drosophila* ovaries, the phagocytic machinery of the follicle cells is essential for the death and removal of germ-line-derived nurse cells[13,14]. Furthermore, in *Drosophila* testes, cyst cells remove excess progenitor germ cells by phagocytosis, and inactivation of the cyst cell genes that mediate phagocytosis prevents death of the progenitor germ cells[16].

During mammalian development, macrophages phagocytose cells undergoing 'programmed cell senescence' (that is, a developmentally programmed cessation of cell proliferation), contributing to the healthy development of multiple tissues[68]. Microglia phagocytose neuronal precursor cells and newly generated neurons in the developing and adult brain to regulate neuronal cell numbers[69–72]. Microglial cell phagocytosis of viable oligodendrocyte progenitor cells regulates myelination during early postnatal development of mice[73,74]. Similarly, numbers of haematopoietic stem cells are regulated by macrophage phagocytosis of these live cells[75,76]. Increased expression of the don't-eat-me signal CD47 on neural and oligodendrocyte progenitor cells can occur in humans as a result of gene copy number variants, and this results in reduced microglial cell phagocytosis of these live progenitor cells, increased brain size (known as macrocephaly) and severe behavioural deficits[77].

Phagoptosis also regulates sperm cell numbers in both males and females. Excess spermatozoa are phagocytosed alive by surrounding epithelial cells in the testis of men and other mammals[78,79]. After insemination, neutrophils and macrophages phagocytose live sperm in the female genital tract, contributing to sperm selection and limiting fertilization[80,81].

Turnover of erythrocytes and neutrophils

Erythrocytes are the most abundant cell in the mammalian body (representing 25% of all host cells in humans), and they turn over every 120 days in humans, resulting in the death of two million erythrocytes per second in humans, which is the highest turnover of any cell type[82]. Erythrocyte turnover and physiological death occurs through phagocytosis of the 'senescent' erythrocytes by macrophages in the spleen, liver and bone marrow[2,3,83]. This phagocytosis of senescent erythrocytes is termed 'erythrophagocytosis', and has been reported to be mediated by the senescent erythrocytes exposing phosphatidylserine and galactose residues, altered CD47 and sialylation, and increased binding of complement and antibodies to erythocytes[2,3,39,83,84]. Depleting macrophages from the body decreases erythrocyte turnover, indicating that macrophages contribute to erythrocyte turnover[3].

Apart from physiological turnover, erythrocytes may die by a process called eryptosis, which is characterized by calcium elevation and phosphatidylserine exposure on the live erythrocytes, inducing their phagocytosis by endothelial cells and macrophages. Eryptosis is a form of cell death by phagocytosis, and is triggered by toxins, drugs, disease, oxidative stress or hyperthermia[82]. For example, *Plasmodium*-infected erythrocytes expose mannose residues on their surface, resulting in their phagocytosis by macrophages, and thus contributing to clearance of the malaria-causing parasite[85]. Thus, both physiological and pathological death of erythrocytes is mediated by phagocytosis, and as

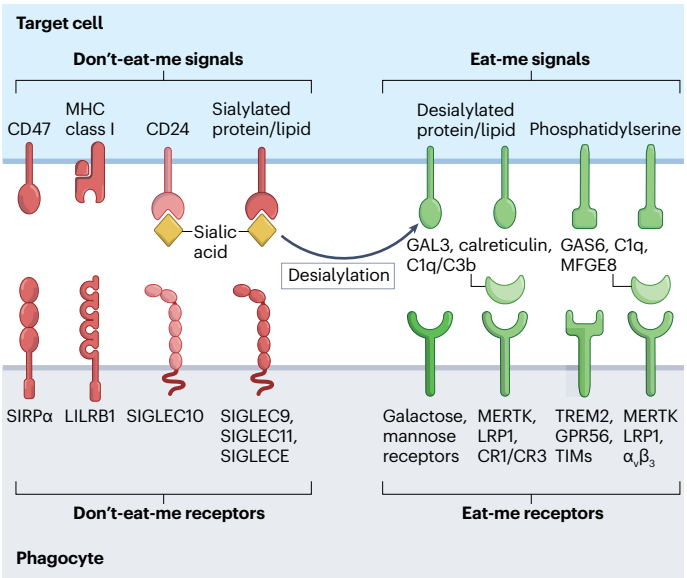

Fig. 2 | Eat-me signals, don't-eat-me signals, opsonins and phagocytic receptors. Don't-eat-me signals include CD47, which can activate signal regulatory protein-α (SIRPα) on phagocytes to inhibit phagocytosis. However, in some circumstances CD47 can increase phagocytosis, possibly due to a change in its conformation or distribution[84]. MHC class I can inhibit phagocytosis by activating LILRB1 (leukocyte immunoglobulin-like receptor subfamily B member 1) on phagocytes. Sialic acid residues on CD24 and other sialylated proteins and lipids act as don't-eat-me signals by activating sialic acid-binding immunoglobulin-type lectin (SIGLEC) receptors on phagocytes[50]. Desialylation (and subsequent modifications) of these glycoproteins and glycolipids reveals galactose, *N*-acetyl-glucosamine and mannose residues, which can act as eat-me signals by activating galactose receptors, mannose receptors or complement receptor 3 (CR3); by binding opsonins, such as galectin 3 (GAL3; which activates MER tyrosine kinase (MERTK)), calreticulin (which activates low-density lipoprotein receptor-related protein 1 (LRP1)) or complement component C1q (which activates LRP1); and by binding C1q or mannose-binding lectin (MBL), which induce C3 cleavage to C3b (that activates CR1 and CR3). The eat-me signal phosphatidylserine directly activates triggering receptor expressed on myeloid cells 2 (TREM2), GPR56 and T cell immunoglobulin and mucin domain (TIM) receptors, and binds opsonins growth arrest specific 6 (GAS6), C1q and milk fat globule-EGF factor 8 (MFGE8) to activate MERTK, LRP1 and vitronectin receptors ($\alpha_v\beta_3$ integrin).

erythrocyte turnover is the highest of any cell type in the body, cell death by phagocytosis represents the most common form of cell death in the body.

Neutrophils turn over at the rate of 0.5–1 million cells per second in humans, live for approximately 1 day and are phagocytosed by macrophages in the bone marrow, spleen and liver[86]. Physiological turnover of neutrophils has been thought to be mediated by apoptosis partly based on the rapid rate of apoptosis in isolated neutrophils. However, overexpression of the anti-apoptotic factor BCL2 in mice has no effect on neutrophil turnover or on phagocytosis of aged neutrophils by macrophages[87]. Macrophages phagocytose aged but viable neutrophils due to calreticulin binding to the desialylated, aged neutrophils[47] — where 'aged' here means 24 h old! Neutrophils can be phagocytosed alive by macrophages in a wide range of circumstances[44,46,47], and this process may contribute to physiological turnover of neutrophils[86]. However, there is evidence of some contribution of apoptosis to the turnover of neutrophils[45], and the quantitative contribution of different forms of cell death to physiological turnover of neutrophils is still unclear.

In conclusion, cell death by phagocytosis is responsible for erythrocyte turnover[82] and may also contribute to neutrophil turnover[86]. As such, cell death by phagocytosis appears to be the most abundant form of cell death in mammals[22].

Immunity by phagocytosis

Neutrophil and macrophage-mediated phagocytosis of live bacteria kills the bacteria, and is a major mechanism of immunity to bacterial infections in animals[66,88]. Such phagocytosis can be driven by scavenger receptors binding to pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), on bacteria[1,88]. Complement components binding to bacteria (or other pathogens) and resulting in deposition of complement opsonins also drives phagocytosis[1,66,88]. In contrast to these innate immune processes, adaptive immunity mediated by phagocytosis occurs when specific antibodies to the pathogen are induced and bind to the pathogen to stimulate phagocytosis via Fc receptors, or to induce complement opsonization via the classical pathway — known in this context as antibody-dependent cellular phagocytosis. Cancer cells and other pathogens can also die as a result of antibody binding to these cells and inducing their phagocytosis[4]. Note, however, that phagocytosis of pathogens by host phagocytes is not always beneficial, as illustrated by macrophage phagocytosis of *Mycobacterium tuberculosis*, which is required for infection of the macrophages and drives the subsequent lung pathology seen in tuberculosis[1].

Neutrophils that have phagocytosed bacteria or cancer cell debris become activated and are then more likely to be phagocytosed alive by dendritic cells, which can then present antigens derived from the bacteria or cancer cells to induce adaptive immune responses[89]. It is tempting to speculate that neutrophil turnover is so rapid because this enables rapid cross-presentation by dendritic cells of antigens originally phagocytosed by neutrophils. Of course, dendritic cells or other antigen-presenting cells can also directly phagocytose live pathogens and present antigens from them to $CD8^+$ T cells to initiate an adaptive immune response[90,91]. Interestingly, when these T cells recognize their specific antigen, they reversibly expose phosphatidylserine on their surface[42]. This phosphatidylserine exposure makes the activated T cells targets for phagocytosis by macrophages, thus limiting the extent of the initial adaptive response and regulating the numbers of remaining memory T cells[92] (Fig. 3). Also, during negative selection in the thymus, $CD8^+$ T cells that recognize self-antigens presented by macrophages expose phosphatidylserine, resulting in their cell death through phagocytosis by the same macrophage presenting the antigen — blocking this process can lead to autoimmunity[93].

Live host cells infected by viruses or bacteria may be phagocytosed by phagocytes, because of exposure of antigens, eat-me signals or opsonins, resulting in cell death by phagocytosis of the infected cell, clearance of the infection and, potentially, an adaptive immune response[94,95]. For example, adenovirus-infected brain cells expose phosphatidylserine and are phagocytosed alive by microglia to clear the infection[96]. As already mentioned above, erythrocytes infected

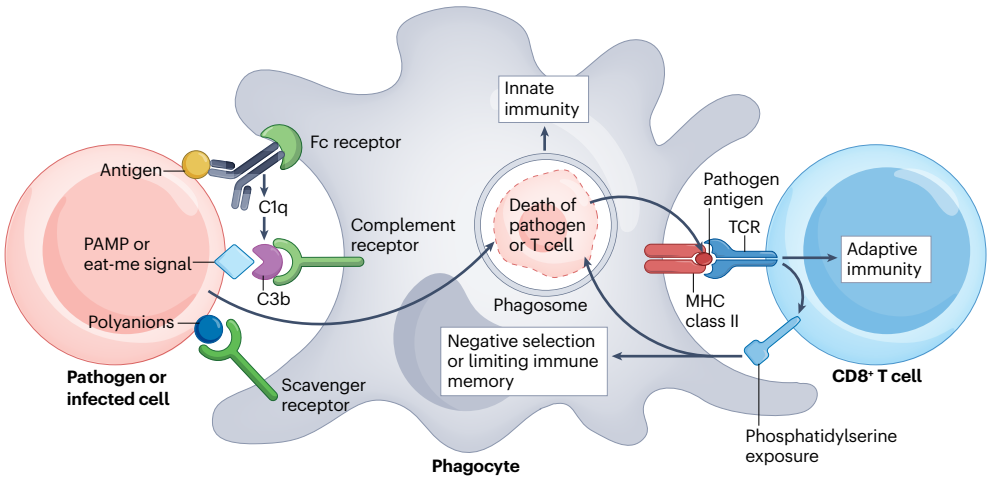

Fig. 3 | Immunity by phagocytosis. Pathogens (or pathogen-infected cells) are recognized and engulfed directly via scavenger receptors, or indirectly via binding IgG antibodies (that activate Fc receptors) or complement components C1q or C3b and/or iC3b (that activate complement receptors (CRs)). Phagocytosis of the pathogen results in cell death by phagocytosis of the pathogen, limiting infection, but may also cause antigen presentation, resulting in adaptive immunity. Antigen-mediated activation of T cell receptors (TCRs) on T cells causes phosphatidylserine exposure on the T cells, which induces their phagocytosis by macrophages. In this way, phagocytosis can limit the number of memory T cells and mediate negative selection of developing thymocytes in the thymus. PAMP, pathogen-associated molecular pattern.

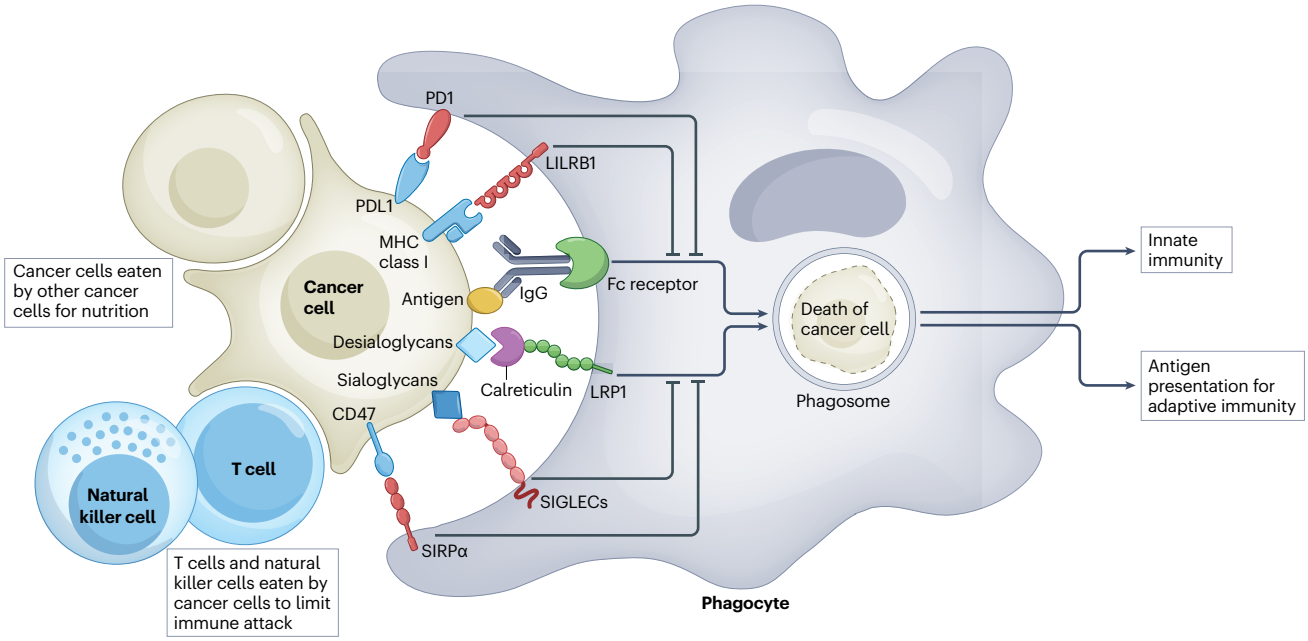

Fig. 4 | Cell death by phagocytosis in cancer. Cancer cells can eat live lymphocytes or other cancer cells for survival or nutrition. Phagocytes can phagocytose live cancer cells via IgG and Fc receptors, or calreticulin and low-density lipoprotein receptor-related protein 1 (LRP1), but this is inhibited by CD47 overexpression on the cancer cell activating signal regulatory protein-α (SIRPα) on the phagocyte to inhibit phagocytosis. Sialylation, CD24, PDL1 and MHC class I may also act as don't-eat-me signals on specific cancer cells. Blocking these don't-eat-me signals with function-blocking antibodies can induce macrophage phagocytosis of the cancer cells and cancer clearance. LILRB1, leukocyte immunoglobulin-like receptor subfamily B member 1; SIGLEC, sialic acid-binding immunoglobulin-type lectin.

by the malaria parasite *Plasmodium falciparum* expose mannose residues of their cell surface, which act as an eat-me signal to promote their removal by splenic macrophages[85]. Macrophage phagocytosis of malaria-infected erythrocytes can further be enhanced by antibody blockade of the don't-eat-me signal CD47, resulting in improved clearance of malaria in mice[97].

Cancer

Cancer cells can be phagocytosed alive by macrophages, resulting in death of the cancer cells, and this appears to be an important mechanism of cancer immunity[4]. Cancer cells can be recognized by phagocytes either via binding of specific antibodies to novel antigens[98] or by innate signals[99], which may include exposure of the eat-me signals phosphatidylserine[100] or calreticulin[101]. Calreticulin and other opsonins may be binding to altered sugar chains on the cancer cell surface[47]. Phagocytosis of cancer cells exposing calreticulin by macrophages or dendritic cells promotes antigen presentation, and therefore an adaptive response to the cancer[48]. Antibodies binding to antigens on cancer cells can induce macrophage phagocytosis of live cancer cells, resulting in death and clearance of the cancer cells, prevented by blocking the phagocytosis[98,102].

In order to prevent macrophage phagocytosis of cancer cells, numerous cancers overexpress CD47, which inhibits phagocytosis by engaging SIRPα on macrophages[4]. Blocking this pathway using antibodies against CD47 or SIRPα can induce clearance of these cancers, and may represent a novel treatment option[4,103]. In some cancers, the expression of other don't-eat-me signals such as CD24 or MHC class I molecules can function to inhibit phagocytosis of the cancer cells, and targeting these pathways can also promote cancer clearance in mouse models[4].

As mentioned earlier, cancer cells can also phagocytose other cancer cells or host cells in a process known as cell cannibalism[28–31]. For example, metastatic melanoma cells (but not primary melanoma cells) phagocytosed live lymphocytes, apparently to limit immune attack and also to provide nutrients for melanoma growth[31]. Cancer cells have also been found to phagocytose natural killer cells, ultimately resulting in the death of these immune cells[33] (Fig. 4).

Neurology

Neurons die by various mechanisms, including microglial phagocytosis of live neurons[104]. Microglia phagocytose stressed, damaged or excess neurons, resulting in neuronal cell death[105]. However, cell death by phagocytosis of neurons is potentially damaging because of the limited neurogenesis that occurs in adults. Microglial phagocytosis of live neurons may contribute to neurological disease, as indicated by the finding that blocking phagocytosis can prevent neuronal loss in animal models of stroke, brain trauma, epilepsy, retinal diseases, Parkinson disease and Alzheimer disease[105–108].

For example, the presence of extracellular amyloid-β or tau, or expression of P301S in neurons, induced reversible exposure of phosphatidylserine on live neurons, and led to microglial phagocytosis of those live neurons; inhibition of phagocytosis prevented neuronal cell loss and death in cultures[24,25,109–113]. In vivo, injection of amyloid-β into the mouse brain induced microglial phagocytosis of neurons and led to the loss of neurons and impaired memory, which was prevented in mice deficient in the $P2Y_6$ receptor required for microglial

Glossary

Cell cannibalism
Cells phagocytosing other cells, which may be dead or alive.

Desialylation
The removal of terminal sialic acid residues from glycoproteins or glycolipids.

Don't-eat-me signals
Molecules on a cell that inhibit a phagocyte eating that cell.

Eat-me signal
A molecule on a cell that induces a phagocyte to eat that cell.

Efferocytosis
Phagocytosis of a cell dying by apoptosis.

Eryptosis
A mechanism of cell death of erythrocytes.

Entotic cell death
The death of a cell that has invaded into another cell by entosis.

Find-me signals
Molecules released from a cell that encourage a phagocyte to chemotactically migrate to the cell.

Haemophagocytosis
The phagocytosis of blood cells.

NADPH oxidase
A membrane-bound enzyme that uses cytosolic NADPH to reduce oxygen to superoxide that is released into phagosomes to kill engulfed cells.

Nurse cells
Specialized cells that support the growth and stability of neighbouring cells.

Opsonins
Normally extracellular molecules that when bound to a cell induce a phagocyte to eat that cell.

Phagocytic receptors
Receptors that directly bind eat-me signals or opsonins and then induce phagocytosis.

Programmed cell removal
The phagocytic removal of cells that may be dead, dying or alive.

Primary phagocytosis
The same as cell death by phagocytosis.

Scavenger receptors
A diverse set of receptors that mediate phagocytosis or endocytosis.

Secondary phagocytosis
Phagocytosis of a dead or dying cell.

Sialic acid-binding immunoglobulin-type lectin (SIGLEC) receptors
A family of receptors binding sialic acid residues.

phagocytosis of neurons[58,114]. Similarly, expression of human P301S TAU in neurons of mice resulted in a loss of neurons and memory that was prevented by crossing these mice with $P2Y_6$ receptor knockout mice[114]. Knockout of the $P2Y_6$ receptor also prevented loss of dopaminergic neurons in the substantia nigra in an LPS-induced model of Parkinson disease[115], and prevented loss of synapses and memory induced by ageing in mice[116] (Fig. 5).

Genome-wide association studies have linked Alzheimer disease risk to variants of many genes regulating microglial phagocytosis[117]. For example, the R47H variant of *TREM2* increases Alzheimer disease risk fourfold, and this variant increases TREM2 activation by phosphatidylserine, resulting in increased microglial phagocytosis of synapses and neurons[118]. TREM2 knockout mice also have less synaptic and neuronal loss during ageing[119], suggesting that TREM2 mediates microglial phagocytosis of live neurons during ageing.

Cell death by phagocytosis of neurons or photoreceptors by microglia has also been shown to contribute to retinal neurodegenerative diseases, such as glaucoma, macular degeneration and retinitis pigmentosa[120,121]. DNA damage within microglia, as occurs in the neurodegenerative disease ataxia telangiectasia, can also induce microglia to phagocytose live neurons[122]. Viral infection of live neurons can induce microglia to phagocytose synapses[123] or neurons[124]. Bacterial infection of microglia can induce the microglia to phagocytose live neurons[125]. Bacterial LPS can also stimulate microglia to phagocytose neurons[24,109,126]. After stroke, delayed neuronal loss can occur in brain areas around, or connected to, the infarct, and there is evidence that cell death by phagocytosis of stressed neurons by microglia contributes to this neuronal loss[106,127–129]. Microglia can also phagocytose activated neutrophils to help resolve brain inflammation, for example after stroke[130].

As discussed previously, microglial phagocytosis of neurons is not normally pathological but, rather, contributes to shaping neuronal circuits. For example, during development, microglial phagocytosis of neurons determines the number of remaining neurons in the sexually dimorphic nucleus of the preoptic area of rats, which subsequently determines sexual preference[131]. A proportion of newborn neurons generated in the neurogenic niches of the dentate gyrus and subventricular zone are phagocytosed alive by microglia, as indicated by the finding that knockout of the microglial phagocytic receptors MERTK and AXL in mice enabled twice as many newly generated neurons to survive, migrate and differentiate[132,133]. This selection of newborn neurons appears to be beneficial, as in a mouse model of amyloidopathy, knockout of MERTK and AXL enabled survival of excessive numbers of newborn neurons in the dentate gyrus and led to seizures that killed the mice[133]. However, excessive phagocytosis of neurons during development may also result in microcephaly[134].

Senescence and ageing

Too much or too little phagocytosis of senescent cells may contribute to ageing[135–137]. The descriptor 'senescent cell' means different things to different people, including 'aged cells', 'cells that have lost some function with age' or 'cells that have stopped proliferation due to telomere erosion or other DNA damage'. As discussed above, neutrophils become 'senescent' after just 24 h in the circulation, and they may then be removed by phagocytosis[86]. By contrast, erythrocytes live, on average, 3 months in the circulation before being phagocytosed as senescent cells by macrophages[2,3,83]. During mammalian development, macrophages phagocytose live cells undergoing programmed cell senescence, thereby contributing to healthy development of multiple tissues[68]. Clearance of senescent cancer cells from the body also can limit cancer development[138].

Senescent cancer cells apparently can become highly phagocytic and phagocytose neighbouring cells alive, resulting in cell death by phagocytosis[139]. By contrast, senescent microglia in aged mice have reduced phagocytosis, potentially contributing to the accumulation of amyloid plaques in Alzheimer disease[140].

The accumulation of senescent cells in aged animals (due to telomere erosion or other DNA damage) is thought to contribute to ageing phenotypes[135,136]. The opsonin MBL preferentially binds to senescent cells, inducing phagocytosis of these, and gene variants of *MBL* are associated with human longevity, suggesting that clearance of senescent cells has a role in longevity[141]. Recently, it was found that senescent cells upregulate their expression of CD47 and CD24 to block phagocytes from phagocytosing the senescent cells[135]. This suggests that it might be

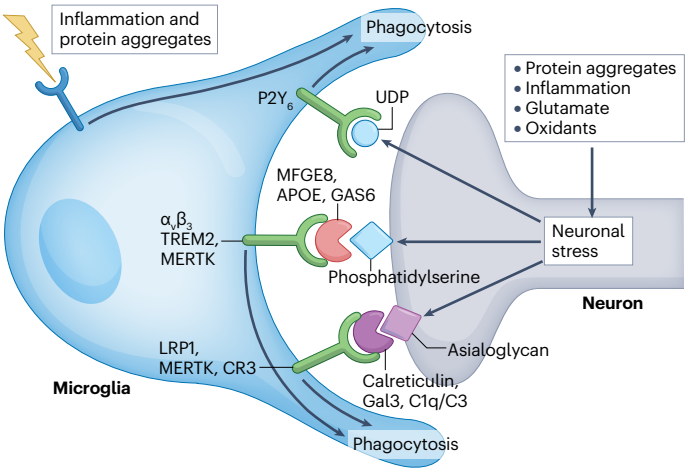

Fig. 5 | Cell death by phagocytosis contributes to neurodegeneration. Neurons stressed by inflammation, protein aggregates, glutamate or oxidants may expose or release eat-me signals, such as phosphatidylserine, calreticulin or UDP, inducing activated microglia to phagocytose the stressed but viable neurons. Phosphatidylserine induces phagocytosis, either via triggering receptor expressed on myeloid cells 2 (TREM2), or milk fat globule-EGF factor 8 (MFGE8) and the vitronectin receptor ($\alpha_v\beta_3$ integrin), or growth arrest specific 6 (GAS6) and MER tyrosine kinase (MERTK). UDP release induces phagocytosis by activating the $P2Y_6$ receptor. Microglia activated by inflammation or protein aggregates express and activate phagocytic receptors and release opsonins and sialidase. Sialidase activates phagocytic receptors, removes the don't-eat-me signal sialic acid and exposes asialoglycans, which bind the opsonins calreticulin, galectin 3 (GAL3) and C1q (which can induce C3 cleavage). Calreticulin and C1q induce phagocytosis via low-density lipoprotein receptor-related protein 1 (LRP1), GAL3 via MERTK and iC3b via complement receptor 3 (CR3).

possible to encourage phagocytes to phagocytose senescent cells and thus clear them from the body as a treatment for ageing, for example by using anti-CD47 antibodies or indeed any antibody that specifically binds to senescent cells.

Anti-CD47 antibodies have been found to be beneficial in animal models of age-related pathologies, including atherosclerosis[137], fibrotic diseases[103] and metabolic disease[136], apparently by inducing clearance of pathological cells[103]. For example, atherosclerotic plaques contain live but dysfunctional macrophages and smooth muscle cells that can be cleared using anti-CD47 antibodies[103,137].

It is tempting to speculate that in future it may be possible to genetically engineer a human phagocyte cell line to phagocytose particular disease-inducing cells (such as cancer, atheroma or senescent cells) when injected into the body. However, for now, it is more feasible to engineer antibodies to encourage endogenous phagocytes to do the same. Indeed, with anti-CD47 and anti-SIRPα antibodies, the era of engineered cell death by phagocytosis is already upon us. The number of cell surface signals regulating phagocytosis is known to be large (about 50 such signals have been described)[36], and this presents new opportunities to broaden and finesse such treatments.

Conclusions

Cell death by phagocytosis is one of the oldest, most common and most important forms of cell death. There is ample evidence that phagoptosis contributes to the death of many cell types — sperm, sperm progenitors, nurse cells, haematopoietic stem cells, oligodendrocyte precursor cells, neuronal precursors, neurons, photoreceptor cells, bacteria, cancer cells, prey cells, virus-infected cells, parasite-infected cells, B cells, T cells, natural killer cells, erythrocytes, neutrophils, macrophages, smooth muscle cells and senescent cells are just some of the cell types that have been looked at. Our current understanding of the mechanisms, extent and significance of cell death by phagocytosis is limited by the paucity of methods to image live cell phagocytosis in vivo, to prevent phagocytosis of specific cells and to quantify the contribution of phagoptosis to cell turnover in health or disease. One approach to the latter might be to transiently induce expression of a tissue-specific marker (such as a fluorescent protein), and then follow the rate of loss of these marked cells. This approach could be combined with the overexpression of inhibitors of phagocytosis in these same cells as well as tracking the uptake of the marked cells into phagocytes. Solving these existing methodological issues may enable the true extent and significance of cell death by phagocytosis to be properly evaluated and facilitate the targeting of this process for disease therapy.

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