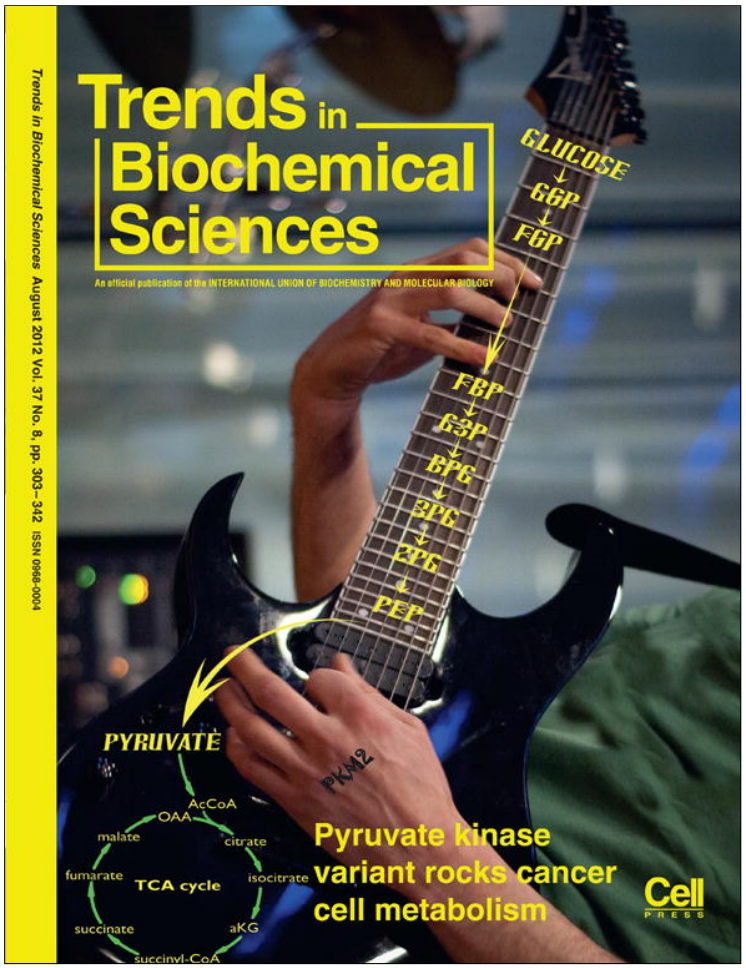

Review

Cell PRESS

Eaten alive! Cell death by primary phagocytosis: 'phagoptosis'

Guy C. Brown and Jonas J. Neher

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

Phagoptosis, also called primary phagocytosis, is a recently recognised form of cell death caused by phagocytosis of viable cells, resulting in their destruction. It is provoked by exposure of 'eat-me' signals and/or loss of 'don't-eat-me' signals by viable cells, causing their phagocytosis by phagocytes. Phagoptosis mediates turnover of erythrocytes, neutrophils and other cells, and thus is quantitatively one of the main forms of cell death in the body. It defends against pathogens and regulates inflammation and immunity. However, recent results indicate that inflamed microglia eat viable brain neurons in models of neurodegeneration, and cancer cells can evade phagocytosis by expressing a 'don't-eat-me' signal, suggesting that too much or too little phagoptosis can contribute to pathology. This review provides an overview of the molecular signals that regulate phagoptosis and the physiological and pathological circumstances in which it has been observed.

Phagoptosis: an invisible form of cell death

Cell death is important in both physiology and pathology: it shapes development, removes excess or defective cells, and protects against pathogens and cancer; but it also contributes to disease. Three main types of cell death have been distinguished: apoptotic, autophagic and necrotic (Box 1). Phagocytosis normally accompanies cell death, but has been regarded as secondary to apoptosis or necrosis (the primary causes of cell death), so it was thought that phagocytes only eat dead cells or cells doomed to die [1,2]. However, it is now clear that phagocytosis can execute death of viable cells, which has been referred to as primary phagocytosis. We propose here that this form of cell death be called phagoptosis, with the defining characteristic that inhibition of phagocytosis prevents cell death. This term is created by combining phago-, which is derived from the ancient Greek 'phagein' meaning to devour, and -ptosis, which is from the ancient Greek 'ptosis' meaning to fall; used here with the connotation of dying; therefore, phagoptosis would connote 'devouring-induced death' or 'death caused by being devoured'. There has previously been no generally accepted name or concept of phagocytic cell death of host cells in the literature, contributing to its relative anonymity and invisibility. Under-recognition of phagocytic cell death might also be because cell culture studies of cell death and disease are usually conducted in the absence of phagocytes, and *in vivo*, the presence of phagocytes and phagoptosis results in no cell corpses left to diagnose. By contrast, phagocytic cell death of pathogens (in contrast to host cells) has been well recognised since the Russian biologist Ilya Ilyich Mechnikov discovered white blood cells phagocytosing bacteria in the 1880s. However, ever since Mechnikov argued passionately that this phagocytosis mediated immune defence and was beneficial, it has been assumed that phagocytosis and phagocytes are always beneficial and avoid eating viable host cells.

Phagoptosis results from the reversible exposure of phosphatidylserine (PS) or other 'eat-me' signals, and/or the loss of 'don't-eat-me' signals, on the surface of an otherwise viable cell, causing its phagocytosis. Such exposure of 'eat-me' signals or loss of 'don't-eat-me' signals can result from physiological activation or a subtoxic insult to the target cell (i.e. the cell that gets eaten) deriving from either the phagocyte or elsewhere. However, in both cases, by definition, inhibition of phagocytosis prevents cell death.

'Eat-me' versus 'don't-eat-me' signalling

The process of phagocytosis is normally initiated by the release of attractive signals (referred to as 'find-me' signals) from the target cell leading to chemotaxis of a nearby macrophage, a type of phagocyte [1]. Upon reaching the target cell, the macrophage recognises cell-surface signals on the target cell, which induce target cell uptake ('eat-me' signals). The best-characterised 'eat-me' signal is the cell surface exposure of the phospholipid PS [1,3,4]. In healthy cells that are not activated, PS is found almost exclusively on the inner leaflet of the plasma membrane because an aminophospholipid translocase uses ATP to pump PS from the outer to inner leaflet (Figure 1). However, a calcium-activated phospholipid scramblase can expose PS on the cell surface by randomising phospholipid distribution between the inner and outer leaflets. PS exposure can occur as a result of: (i) calcium elevation, which stimulates the scramblase and inhibits the translocase; (ii) ATP depletion, which inhibits the translocase; (iii) oxidative stress, which stimulates the scramblase and inhibits the translocase; (vi) fusion of intracellular vesicles with plasma membrane; (iv) necrosis, due to plasma membrane rupture, calcium elevation or ATP depletion; or (v) apoptosis, when PS exposure occurs secondary to calcium elevation, ATP depletion, oxidative stress or vesicle fusion [5–7].

Although displaying PS has previously been regarded as an early sign of apoptotic cell death, it is now clear that PS exposure can occur on the surface of viable cells independent of apoptosis [5,8–10], and can be reversible [11–15]. PS exposure on viable cells can lead to the phagocytosis of these cells when in the presence of macrophages which are

Corresponding author: Brown, G.C. (gcb@mole.bio.cam.ac.uk).
Keywords: phagocytosis; apoptosis; cell death; turnover; inflammation; clearance.

Box 1. What are the major physiological forms of cell death?

Three main types of cell death have been distinguished: apoptotic, autophagic and necrotic [67]. Apoptosis is mediated by caspases and/or Bcl-2 homologous proteins such as Bax and Bak; autophagy is mediated by autophagic vesicles delivered into lysosomes; and necrosis is mediated by rupture of the plasma membrane. However, apoptosis, autophagy and necrosis can occur before, during or after death commitment without being the cause of death. Thus, it is useful to distinguish between autophagy and autophagic cell death, the latter being cell death caused by autophagy; and between primary necrosis and secondary necrosis, the former being cell death caused by necrosis and the latter being necrosis caused by some other form of cell death. Similarly, primary phagocytosis (i.e. phagoptosis) is cell death caused by phagocytosis, whereas secondary phagocytosis is phagocytosis caused by some other form of cell death. Apoptotic processes, including activation of caspases and proapoptotic Bcl-2 homologous proteins, can occur in the absence of cell death, or secondarily to some other form of cell death [54]. Thus, it is important to define apoptotic cell death as cell death that is preventable by inhibition of caspases, knockout of *Bax and Bak* and/or overexpression of anti-apoptotic Bcl-2 homologous proteins. However, in *C. elegans*, developmentally programmed death of certain cells can be prevented by inhibiting either apoptosis or phagocytosis [34,35]. Thus, in certain conditions, a low level of apoptosis might be insufficient to cause cell death by itself, but cause sufficient exposure of 'eat-me' signals to cause phagoptosis [62]. Increasing levels of cellular stress might cause: adaptation, phagoptosis, apoptosis and necrosis, respectively (Figure 4).

The relative amounts of different forms of cell death are hard to quantify. However, mice lacking key components of apoptosis can develop almost normally and have normal cell turnover and organ functions [50,68], with a couple of exceptions: there is an expansion in the T and B cell populations, particularly memory cells, and expansion of a subset of periventricular neurons derived from neural stem/progenitor cells, but only in the *Bax/Bak* double knockout mice [50]. These cell populations expand with age, indicating that apoptosis contributes to their turnover (but not that of any other cells). T cell and B cell turnover in humans is about 1000 and 500 cells per second, respectively, and turnover is faster in memory cells than in naïve cells [69,70]. Turnover of neural progenitors in the adult brain is likely to be lower than this (as total estimated neurogenesis is less than 1 cell per second), so the overall contribution of apoptosis to physiological turnover is estimated to be about 10^3 cells per day.

The rate of cornification of keratinocytes is about 40 000 per second in humans [71,72]. Cornification is a unique mechanism of cell death, unrelated to any other, and is mediated in part by transglutaminases and nonapoptotic caspase-14 [67,68,72]. The intestinal epithelium is shed by a variety of mechanisms at a rate of about 80 000 cells per second in humans [68]. Much of this death probably occurs after shedding from the epithelial layer into the gut, but part of the death occurs prior to shedding, and part of this appears to be necrotic. This is the highest known contribution of necrosis to physiological cell death/turnover, therefore, we can estimate this contribution as in the order of 10^4 cells per second. There is no known contribution of autophagic cell death to physiological turnover of cells. The highest known rates of cell death/turnover in the body are for erythrocytes and neutrophils: 2×10^6 and 0.5×10^6–2×10^6 cells per second respectively in humans [2,44]. This turnover appears to be due to phagoptosis, therefore, this is apparently by far the most prevalent physiological form of cell death in the adult human body (Table I). However, cell death by necrosis, apoptosis and autophagy can increase during infection and pathology.

Table I. Rough estimates of the physiological rates of cell turnover by different forms of cell death in humans

Type of cell death	Cells	Rate (thousands of cells/second)
Phagoptosis	Erythrocytes Neutrophils	2000 500–1000
Shedding	Enterocytes	80
Cornification	Keratinocytes	40
Necrosis	Enterocytes	10
Apoptosis	T cells and B cells	1
Autophagy		None known

competent in binding PS and phagocytosing such cells. For example, galectins induce PS exposure on the surface of activated neutrophils, which is fully reversible if the galectin is removed and does not lead to autonomous cell death [13]. However, if competent macrophages are present at the time of PS exposure then the neutrophil is phagocytised and killed [8,9,14,15].

Blood cells such as neutrophils, platelets, monocytes and lymphocytes are activated by pathogens, damage or antigens. This activation is accompanied by PS exposure on the surface of the activated but viable cells [8,10,11]. PS is also exposed during differentiation of reticulocytes, monocytes, B cells and skeletal muscle [8,30]. PS exposure on viable cells has been proposed to regulate diverse processes including cellular trafficking, fusion and adhesion [10,11]. However, because PS exposure promotes the phagocytosis of these cells, it might also function to limit the lifetime of the activated state. This might be particularly important in the blood because PS exposure is thrombogenic, catalysing blood clotting, and thus such cells need to be cleared as rapidly as possible.

PS recognition by macrophages depends on a range of different receptors, which vary with activation state (Figure 2). For example, resting macrophages express the PS receptors Tim4 (T-cell immunoglobulin- and mucin-domain-containing molecule), stabilin-1, stabilin-2 and BAI1 (Brain-specific angiogenesis inhibitor 1) [21,31,32]. By contrast, activated macrophages upregulate the key PS-binding protein MFG-E8 (milk fat globule EGF-like factor-8, also known as lactadherin, SED1) and its receptor (the vitronectin receptor, an $\alpha_v\beta_{3/5}$ integrin), and the Mer receptor tyrosine kinase (MerTK), which recognises PS or other eat-me signals through the bridging molecules Gas-6 (Growth arrest-specific 6), protein S, galectin-3, tubby and Tulp1 (Tubby-related protein 1) [1]. The PS-binding protein annexin (Anx)A1 is released by activated neutrophils and macrophages, and mediates phagocytosis of activated neutrophils by binding both PS on their surface and formyl peptide receptors on bone marrow-derived macrophages [14,33].

Whether exposure of PS by itself is sufficient for recognition and removal depends on the cell type and conditions. Addition of purified PS to three different cell lines results in a transient elevation of PS on the surface of viable cells, which promotes their phagocytosis, but once the PS has been internalised, there is no further phagocytosis and the cells remain viable [3]. However, constitutive PS-exposure on lymphoma cells (induced by overexpression of the scramblase TMEM16F) is insufficient for phagocytosis [22]. Surface exposure of PS can be insufficient for phagocytosis either because the cells display 'don't-eat-me' signals, or because some cells require

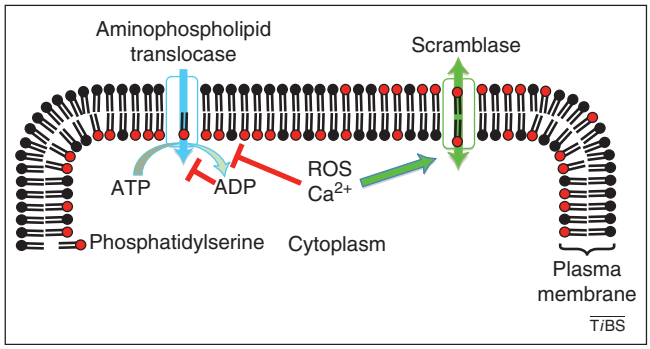

Figure 1. Mechanisms of phosphatidylserine (PS) exposure. The aminophospholipid translocase uses ATP to pump PS from the outer to inner leaflet of the plasma membrane, whereas the calcium-activated phospholipid scramblase can cause PS exposure by randomising phospholipid distribution. PS exposure can occur as a result of: elevated levels of calcium or reactive oxygen species (ROS), which both stimulate the scramblase and inhibit the translocase, or ATP depletion, which inhibits the translocase.

PS oxidation, a PS-binding adaptor protein or other co-stimulatory signals to induce their phagocytosis [1,14,23].

Cell surface calreticulin (CRT) is the second major 'eat-me' signal (Figure 2), inducing phagocytosis of both apoptotic and viable cells via activation of lipoprotein receptor-related protein (LRP) on the phagocyte [4]. Most CRT acts as a chaperone in the endoplasmic reticulum (ER), but ER stress can cause exocytosis of CRT [16]. Once at the cell surface, CRT promotes phagocytosis and antigen presentation by the phagocyte [16]. By contrast, when PS induces phagocytosis, it suppresses antigen presentation and inflammation [1,2]. CRT is constitutively displayed on neutrophils [17] and many cancers [18], requiring strong exposure of 'don't-eat-me' signals to prevent phagoptosis [18]. The means by which CRT is tethered to the cell surface are poorly characterised, but it can bind a variety of surface proteins, PS, or complement C1q on target cells, and activates phagocytosis via LRP on phagocytes [4,16,19]. Complement can also induce phagoptosis of pathogens and host cells by complement receptors, whereas antibodies bound to target cells induce phagocytosis via Fc receptors on phagocytes. Interestingly, there can be endogenous antibodies to PS present in normal plasma that induce phagocytosis of PS-exposed cells [20].

Many cells actively protect themselves from phagocytic removal by displaying 'don't-eat-me' signals. These include CD47, CD200, CD31 and plasminogen activator inhibitor (PAI)-1 (Figure 3). CD47 is expressed on the surface of most cells, and blocks phagocytosis by activating the receptor signal-regulatory proteinα (SIRPα) on the phagocyte; disruption of this 'don't-eat-me' system results in phagoptosis of viable cells [4,24–26]. Sialic acid modifications on cell surface glycoproteins or glycolipids can act as a 'don't-eat-me' signal by a preventing complement C3b and C1q binding and/or their ability to induce phagocytosis [27]. Apoptotic cells can lose sialic acid residues from the surface, and removing sialic acid residues by adding neuraminidase is sufficient to induce phagocytosis of viable lymphoblasts by macrophages [28]. Siglec-11 (sialic acid binding Ig-like lectin 11) is a receptor on microglia (brain macrophages), which binds polysialylated proteins on the surface of neurons, resulting in inhibition of both inflammation and phagocytosis [29]. Transfection of mouse microglia with siglec-11 reduces their spontaneous phagocytosis of viable neurons; this effect is dependent on the presence of polysialylated proteins on the surface of neurons [29]. Desialylation induced complement binding to neurons and microglial phagocytosis via complement receptor 3 (CR3) [27].

Thus, whether a cell is phagocytosed or not depends on its net exposure of 'eat-me' and 'don't-eat-me' signals, as well as the expression of the corresponding receptors on the phagocytes it encounters or recruits. The following sections focus on the biological systems where phagoptosis has been observed.

Phagoptosis in worms and flies

Caenorhabditis elegans, *Drosophila melanogaster* and rodents (mice and rats) are the main model organisms for studying cell death, and thus most of the data available are from these organisms. In *C. elegans*, loss-of-function mutants in *ced-1* or other cell-engulfment genes results in the survival of some cells programmed to die during development. Similarly, weak loss-of-function mutations in *ced-3* or other apoptosis genes increases the number of surviving cells. However, combining both types of mutation, resulting in weakened apoptosis and reduced phagocytosis, causes a dramatic increase in cells surviving development, indicating that both apoptosis and phagoptosis contribute to developmental programmed cell death [34,35]. Similarly, inactivation of cell-engulfment genes prevents cell death induced by certain subtoxic insults or mutations [36]. This implies that weak caspase activation or other cell stressors might be insufficient to induce autonomous cell death, but by causing PS exposure they could be sufficient to promote phagoptosis. In support of this idea, PS exposure is normally reversed by the ATP-dependent translocase tat-1; disruption of *tat-1* exposes PS on the surface of viable cells and is sufficient to induce their phagocytosis via the ced-1 pathway [37]. Phagocytosis of PS-exposed cells is downregulated by srgp-1 (SLIT-ROBO Rho GTPase-activating protein 1), and knockout of this gene in *C. elegans* not only increases phagocytosis of apoptotic cells but also promotes the phagoptosis of subtoxically stressed cells [38].

Developmentally programmed cell death in *D. melanogaster* is induced by expression of Head involution defective (Hid), Reaper (Rpr) and Grim proteins, which bind a caspase inhibitor (DIAP1, Drosophila Inhibitor of apoptosis) and thereby activate caspases to induce apoptosis. Knockout of these three IAP antagonists or global caspase inhibition prevents all apoptosis that occurs during development. However, it does not prevent phagocytosis of most cells that are lost during developmental programmed cell death [39]. This suggests that apoptosis is not required for developmentally programmed cell death in *Drosophila*, but phagocytosis might be required. One mechanism through which *Drosophila* phagoptosis is mediated is though the ER protein pretaporter, which acts as an 'eat-me' signal and ligand for draper (a homologue of ced-1). Expression of pretaporter on the surface of cells is sufficient to induce phagoptosis of viable cells [40].

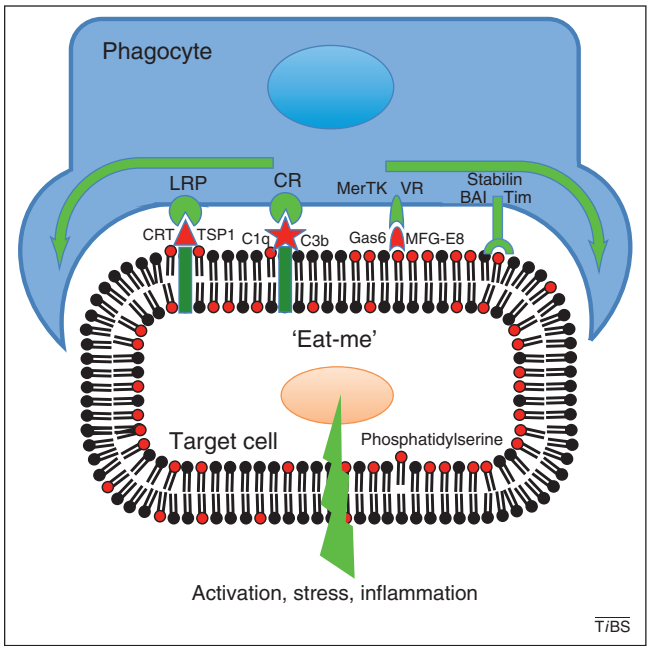

Figure 2. 'Eat-me' signalling. As a result of activation or stress, cells can expose a variety of 'eat-me' signals, including: calreticulin (CRT), thrombospondin 1 (TSP1), complement factors C3b and C1q, and phosphatidylserine (PS). CRT and TSP1 activate phagocytosis via lipoprotein receptor-related protein (LRP) and other receptors on the phagocyte. C3b and C1q activate via complement receptors (CRs), and phosphatidylserine activates either via directly binding receptors such as stabilin, Tim (T-cell immunoglobulin- and mucin-domain-containing molecule) and BAI or via binding adaptor proteins such Gas6 (Growth arrest-specific 6) and MFG-E8 (milk fat globule EGF-like factor-8), which activate phagocytosis via Mer tyrosine kinase (MerTK) and the vitronectin receptor (VR), respectively. Inflammation promotes phagocytosis by phagocytes by activating the phagocytic machinery; increasing expression and/or release of receptors, adaptors and complement; and release of inflammatory factors (such as reactive oxygen species) that induce the target cells to expose 'eat-me' signals.

Cells of different genotypes and/or phenotypes can compete for growth in a tissue. In *Drosophila*, such competition appears to be mediated by phagoptosis of neighbouring cells. Knockout of phagocytic genes such as *draper* and *wasp* prevents competition, whereas activation of phagocytic genes enhances competition by promoting phagoptosis [41].

Erythrocytes

Erythrocytes (red blood cells) are produced in the bone marrow at a rate of about 2 million per second in humans. They live in the circulation for about 120 days, and are then phagocytised by macrophages in the spleen, liver and bone marrow, at the same rate as they are produced [31,42]. This rate of cell death, mediated by erythrophagocytosis, appears to be the highest in the body. Erythrocytes do not undergo apoptosis. Old erythrocytes are preferentially phagocytosed because they express 'eat-me' signals, including PS and altered Band 3 transporter, which is recognised by endogenous IgG. Phagocytosis is inhibited by expression of the 'don't-eat-me' signal CD47 on the erythrocyte surface, which acts on macrophage SIRPα receptors; loss of CD47 is sufficient to induce rapid phagocytosis of erythrocytes *in vivo* [42,43]. Expression of CD47 is reduced in old erythrocytes, contributing to their elimination [44]. Depletion of body macrophages results in increased survival of old erythrocytes [44], indicating that phagoptosis contributes to erythrocyte turnover.

Erythrocytes that have been subtoxically stressed by deformation, oxidation or *in vitro* storage expose PS, which might contribute to their turnover. Clearance of PS-exposed erythrocytes is mediated partly by PS-receptors stabilin-1 and stabilin-2 in the liver, and knockdown of these receptors blocked phagocytosis and delayed the elimination of stressed erythrocytes *in vivo* [31].

Neutrophils and platelets

Neutrophils, the most abundant type of white blood cell, are produced in the bone marrow at a rate of about 0.5–1 million per second in humans, live for about 5 days, and are then phagocytised by macrophages in the spleen, liver and bone marrow [2]. However, in contrast to erythrocytes, younger neutrophils are as likely to be phagocytised as older neutrophils. Also in contrast to erythrocytes, neutrophils undergo spontaneous apoptosis when isolated. However, transgenic mice overexpressing antiapoptotic Bcl-2 have unchanged rates of neutrophil turnover and phagocytosis, even though neutrophil apoptosis is completely blocked, so neutrophil turnover appears to be independent of apoptosis [45].

PAI-1 acts as a 'don't-eat-me' signal on neutrophils: knockout of PAI-1 caused increased phagocytosis of viable neutrophils by macrophages in culture and *in vivo*, which is reversed by adding back PAI-1 protein, which binds to the surface of the neutrophils [46]. PAI-1 blocking antibodies also induce phagocytosis of viable neutrophils [46]. CRT acts as the main 'eat-me' signal driving phagocytosis (via LRP) when PAI-1 or CD47 is blocked, and PAI-1 might inhibit the interaction between CRT and LRP [46]. Neutrophils are the only leukocytes to expose CRT constitutively [17], which might contribute to their rapid physiological turnover and explain why they can be depleted by CD47-blocking antibodies *in vitro* [46] and *in vivo* [47].

Viable neutrophils can be stimulated to expose PS, thereby inducing phagocytosis by macrophages [9,13]. Activated neutrophils generate oxidised PS and lyso-PS (PS missing one acyl chain), which are more potent 'eat-me' signals that induce phagoptosis of activated, viable neutrophils *in vitro* and *in vivo* to terminate the inflammatory response [23,48]. Activated macrophages can also induce PS exposure on viable neutrophils (independent of apoptosis), which then promotes their phagoptosis via PS-binding proteins MFG-E8 and AnxA1, the latter promoting phagocytosis via formyl peptide receptor-like 1 (FRPL-1) [14]. *AnxA1*$^{-/-}$ mice have a defect in clearance of neutrophils in the bone marrow, resulting in an increased density of viable and PS-exposed neutrophils in the bone marrow [33].

Neutrophils are also themselves avid phagocytes, but they only eat small cells such as bacteria. However, they do eat activated platelets *in vitro* and *in vivo* via platelet surface PS and P-selectin, thus downregulating blood clotting via phagocytosis [49]. Physiological platelet turnover is also mediated by macrophage phagocytosis and regulated by CD47 expression on platelets, such that platelets lacking CD47 are rapidly cleared [24].

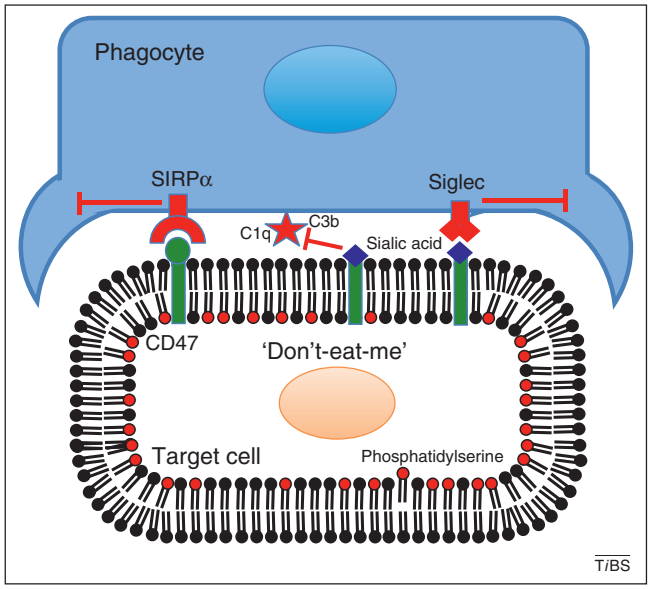

Figure 3. 'Don't-eat-me' signalling. Cells can expose 'don't-eat-me' signals to block their phagocytosis, including: CD47, which binds phagocyte SIRPα (signal-regulatory protein α); and sialic acid residues, which block phagocytosis by binding phagocyte siglec (sialic acid binding Ig-like lectin) and preventing binding of the 'eat-me' signals complement C1q and C3b Cells can lose these 'don't-eat-me' signals when aged, stressed or infected.

T cells

Adaptive immunity is mediated in part by the recognition of novel antigens by an antigen-specific T cell population, which then becomes activated, proliferates, and then contracts leaving memory T cells. Antigen recognition causes PS exposure on the surface of activated T cells [11]. T cell surface PS is recognised by the PS receptor Tim-4, mediating phagoptosis of these cells and thus the contraction phase of the adaptive response [32]. During immunisation with antigen or during infection with influenza virus, blocking Tim-4 on phagocytes increases the expansion and decreases the contraction of antigen-specific T cells, resulting in an increase in subsequent immune responses to those antigens. Conversely, transgenic phagocyte-specific overexpression of *Tim-4* in mice reduces the number of antigen-specific T cells that remain after immunisation, resulting in reduced secondary T cell responses. *In vitro*, expressing *Tim-4* in phagocytes decreases the number of antigen-specific T cells by specifically phagocytising antigen-activated T cells that are capable of proliferation and therefore viable [32]. Similar to erythrocytes, neutrophils and platelets, CD47 acts as 'don't-eat-me' signal on T cells and regulates T cell turnover, therefore, T cells lacking CD47 are readily eliminated by phagocytosis [25]. However, *Bax*/*Bak* double knockout mice, which cannot perform apoptosis, have a large progressive expansion of memory T and B cells [50], indicating that apoptosis also contributes to their turnover (Box 1).

Hemophagocytosis, entosis and cell cannibalism

Hemophagocytosis is a clinical condition, found in many infectious and inflammatory disorders, in which activated macrophages engulf apparently viable blood cells, resulting in reduced white or red cell count (cytopenia). Interferon (IFN)-γ, and possibly other cytokines, appear to drive hemophagocytosis during infection by directly stimulating macrophages to phagoptose blood cells [51]. Macrophages consume blood cells in the absence of inflammation (see above), but inflammation increases both the phagocytic capacity of macrophages and the PS exposure of leukocytes, which potentially explains the inflammation-induced hemophagocytosis and cytopenia.

At various times, living or partly intact cells have been reported within other (non-phagocyte) cells, and this 'cell-in-cell' phenomenon has been called emperipolesis, cell cannibalism or entosis. The cell-in-cell state is relatively common in malignant cancers. Entosis is the process by which tumour cells invade each other when detached from extracellular matrix, which can result in either survival or death of the invading cell by lysosomal digestion [52]. Some metastatic cancer cells phagocytise living leukocytes, particularly T cells, perhaps to protect and feed themselves [53]. However, natural killer (NK) cells can enter target tumour cells, leading to either target cell death or self-destruction within tumour cells via apoptosis [54]. Mormone *et al.* [55] have reported that immortalised lymphoblasts from patients with Huntington's disease actively phagocytosed each other, and they called this phenomenon 'cell cannibalism'. However, it is generally unclear whether inhibition of phagocytosis would prevent death in these cell-in-cell phenomena.

Neurons and inflammatory neurodegeneration

Phagoptosis of neurons in the brain is potentially detrimental to brain function because of the limited capacity to replace such neurons. However, viable neurons are phagocytised by microglia (brain macrophages) that have been activated by amyloid-β or lipopolysaccharide (LPS) [15,56,57]. Imaging of such inflamed glial–neuronal cultures shows microglia phagocytising large numbers of apparently healthy neurons. Inflammatory activation caused the microglia to become highly phagocytic and release the phagocytic adaptor protein MFG-E8 and reactive oxygen and nitrogen species, which induce reversible PS exposure on neurons and lead to neuronal phagocytosis by microglia [15,56,57]. Stressed but viable neurons can reversibly expose PS, which only results in phagocytosis if activated microglia are present at the time of PS exposure [15]. During inflammation, the PS exposed on neurons is bound by MFG-E8, which induces phagocytosis via the microglial vitronectin receptor. Thus, blocking exposed PS, MFG-E8 or the vitronectin receptor prevents all neuronal loss without inhibiting inflammation, leaving viable neurons. Inflammatory neuronal loss is absent in neuronal–microglial co-cultures from MFG-E8 knockout mice, which have a normal inflammatory response; neuronal loss can be reconstituted by adding purified MFG-E8 to the cultures. LPS injection into the striata of the brains of rats and mice *in vivo* causes strong microglial inflammation and neuronal loss, but this neuronal loss is much reduced in MFG-E8 knockout mice, or by co-injection of a vitronectin receptor inhibitor, resulting in the survival of viable neurons [57]. Thus, it appears that inflammation in the brain can cause microglia to eat viable neurons, and this can be prevented by blocking phagocytic signalling.

Microglia can also eat apoptotic neurons, which is potentially beneficial because it reduces debris and inflammation. However, it appears that inflammatory activation of microglia impairs their ability to discriminate between apoptotic and viable neurons for phagocytosis, resulting in phagoptosis during inflammation [58].

Microglia are also known to kill neurons, potentially through phagoptosis, as a normal part of development. Specifically, microglia kill developing neurons in the cerebellum [59] and the hippocampus [60], by a process requiring microglial contact and prevented by blocking the phagocytic receptor CR3 [60]. Microglia can also spontaneously phagocytise viable neurons and neuronal processes in culture, which is increased by removal of surface sialic acid residues and decreased by transfecting microglia with the sialate receptor siglec-11 [29]. Desialylated neurons bind complement C1q, which activates their phagocytosis via microglial CR3 [27]. During development, microglia are involved in synaptic pruning (phagocytosis of synapses) [61], which is partly mediated by C1q; so it is tempting to speculate that phagocytosis of neurons is an extension of this activity.

A major cause of frontotemporal degeneration (FTD) is heterozygous, inactivating mutation in the progranulin gene. Progranulin was recently found to inhibit phagocytosis of apoptotic and/or PS-exposed cells in culture and *in vivo*, suggesting that neuronal loss in FTD is caused by phagoptosis that progranulin normally suppresses [62]. Polymorphisms in the progranulin gene are associated with Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, raising the possibility that premature phagocytosis of neurons and synapses contributes to neurodegeneration [63].

Cancer

Cancer development might be limited by antibody- and complement-dependent phagocytosis of cancer cells, or by induction of 'eat-me' signals by macrophages and T cells. Oncogene-induced senescence might also induce macrophage phagocytosis of tumour cells [64]. Perhaps unsurprisingly then, most human cancers have been found to overexpress the 'don't-eat-me' signal CD47, and the level of CD47 expression correlates with tumourigenicity in mice and mortality in humans [47,65]. Overexpressing CD47 in leukaemic cells increases tumourigenicity and inhibits phagocytosis by macrophages *in vitro* and *in vivo* [47,65]. Antibodies against CD47 induce phagocytosis of leukaemic cells by macrophages *in vitro* and *in vivo*, and thereby completely clear multiple types of leukaemia from mice [26,65,66]. The 'eat-me' signal that appears to drive this phagoptosis once CD47 is neutralised is CRT, which is exposed at high levels on cancer cells [18].

Concluding remarks

Phagoptosis is cell death caused by phagocytosis by another cell, and therefore is prevented by blocking phagocytosis or phagocytic signalling. Whether a cell is phagocytosed or not depends on its net exposure of 'eat-me' and 'don't-eat-me' signals, as well as the expression of the corresponding receptors on the phagocytes it encounters. There has been no common name or concept of phagocytic cell death in the past, which has contributed to its relative invisibility, so we propose here that phagocytic cell death be known as phagoptosis. Far from being a rare and esoteric event, phagoptosis is widespread amongst multicellular organisms, is a major homeostatic mechanism throughout life, and can cause several pathological conditions when it goes awry. Phagoptosis can occur as one of several possible responses to cellular stress. Depending on the level of stress, these responses include: adaptation, phagoptosis, apoptosis and necrosis (Figure 4). Future work will further define the conditions under which phagoptosis occurs and which components of this pathway can be targeted therapeutically to treat disease (Box 2).

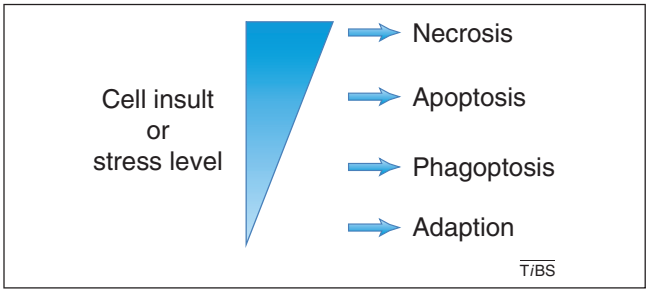

Figure 4. Increasing levels of cellular stress or insult can cause: adaptation, phagoptosis, apoptosis and necrosis, respectively.

Box 2. Outstanding questions

- How do different 'eat-me' and 'don't-eat-me' signals and their receptor signalling interact to determine whether or not target cells are engulfed by phagocytes?
- Which signals, adaptors, and receptors are important for phagoptosis of particular cells in particular conditions?
- Can we develop specific drugs to block 'eat-me' and 'don't-eat-me' receptors?
- We know that phagoptosis contributes to the physiological turnover of blood cells, but does it contribute to physiological turnover of cells in other tissues?
- Can we find better ways of assaying or visualising phagoptosis in culture and *in vivo*, or find biomarkers for phagoptosis that can be used on patient tissue samples?
- What is the relation between apoptosis and phagoptosis? Can subtoxic apoptosis induce phagoptosis in mammalian cells? Can phagoptosis induce apoptosis in the engulfed cell?
- How are cells actually killed during phagoptosis? Is it by fusion of the phagosome containing the cell with lysosomes and subsequent digestion? Or do oxidants from the NADPH oxidase participate during engulfment? Why is the engulfed rather than the engulfing cell killed?
- What are the immunological consequences of phagoptosis? Does it induce or supress antigen presentation, and in which circumstances? What roles does it play in the immunology of T and B cells?
- What role does phagoptosis play in the resolution of inflammation? Can resolution be enhanced by stimulating phagoptosis?
- Are transplanted stem cells restricted by phagoptosis? Could they be protected by expressing 'don't-eat-me' signals?
- Can increased phagoptosis clear cancers?
- Does phagoptosis play roles in aging, diabetes and atherosclerosis?

Acknowledgements
Research related to this review was supported by the Wellcome Trust (Grant RG50995). Many thanks to: Aviva Tolkovsky, Michael Fricker, Urte Neniskyte and Vilma Borutaite for discussion and contributions to these ideas and findings.

References

1 Ravichandran, K.S. (2011) Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. *Immunity* 35, 445–455
2 Bratton, D.L. and Henson, P.M. (2011) Neutrophil clearance: when the party is over, clean-up begins. *Trends Immunol.* 32, 350–357
3 Fadok, V.A. *et al.* (2001) Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *J. Biol. Chem.* 276, 1071–1077
4 Gardai, S.J. *et al.* (2005) Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell* 123, 321–334
5 Tyurina, Y.Y. *et al.* (2007) Nitrosative stress inhibits the aminophospholipid translocase resulting in phosphatidylserine externalization and macrophage engulfment: implications for the resolution of inflammation. *J. Biol. Chem.* 282, 8498–8509
6 Mirnikjoo, B. *et al.* (2009) Suicidal membrane repair regulates phosphatidylserine externalization during apoptosis. *J. Biol. Chem.* 284, 22512–22516
7 Mirnikjoo, B. *et al.* (2009) Mobilization of lysosomal calcium regulates the externalization of phosphatidylserine during apoptosis. *J. Biol. Chem.* 284, 6918–6923
8 Callahan, M.K. *et al.* (2003) Phosphatidylserine expression and phagocytosis of apoptotic thymocytes during differentiation of monocytic cells. *J. Leukoc. Biol.* 74, 846–856
9 Dias-Baruffi, M. *et al.* (2003) Dimeric galectin-1 induces surface exposure of phosphatidylserine and phagocytic recognition of leukocytes without inducing apoptosis. *J. Biol. Chem.* 278, 41282–41293
10 Elliott, J.I. *et al.* (2005) Membrane phosphatidylserine distribution as a nonapoptotic signaling mechanism in lymphocytes. *Nat. Cell Biol.* 7, 808–816
11 Fischer, K. *et al.* (2006) Antigen recognition induces phosphatidylserine exposure on the cell surface of human CD8+ T cells. *Blood* 108, 4094–4101
12 Balasubramanian, K. *et al.* (2007) Regulated externalization of phosphatidylserine at the cell surface: implications for apoptosis. *J. Biol. Chem.* 282, 18357–18364
13 Stowell, S.R. *et al.* (2009) Galectin-1 induces reversible phosphatidylserine exposure at the plasma membrane. *Mol. Biol. Cell* 20, 1408–1418
14 Jitkaew, S. *et al.* (2009) Induction of caspase- and reactive oxygen species-independent phosphatidylserine externalization in primary human neutrophils: role in macrophage recognition and engulfment. *J. Leukoc. Biol.* 85, 427–437
15 Neher, J.J. *et al.* (2011) Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. *J. Immunol.* 186, 4973–4983
16 Panaretakis, T. *et al.* (2009) Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. *EMBO J.* 28, 578–590
17 Ghiran, I. *et al.* (2003) Calreticulin is at the surface of circulating neutrophils and uses CD59 as an adaptor molecule. *J. Biol. Chem.* 278, 21024–21031
18 Chao, M.P. *et al.* (2010) Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci. Transl. Med.* 2, 63ra94
19 Païdassi, H. *et al.* (2011) Investigations on the C1q-calreticulin-phosphatidylserine interactions yield new insights into apoptotic cell recognition. *J. Mol. Biol.* 408, 277–290
20 Kim, J. (2010) Identification of a human monoclonal natural IgM antibody that recognizes early apoptotic cells and promotes phagocytosis. *Hybridoma* 29, 275–281
21 Park, D. *et al.* (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* 450, 430–434
22 Segawa, K. *et al.* (2011) Constitutive exposure of phosphatidylserine on viable cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19246–19251
23 Borisenko, G.G. *et al.* (2004) Milk fat globule epidermal growth factor 8 (MFG-E8) binds to oxidized phosphatidylserine: implications for macrophage clearance of apoptotic cells. *Cell Death Differ.* 11, 943–945
24 Olsson, M. *et al.* (2005) Platelet homeostasis is regulated by platelet expression of CD47 under normal conditions and in passive immune thrombocytopenia. *Blood* 105, 3577–3582
25 Blazar, B.R. *et al.* (2001) CD47 (integrin-associated protein) engagement of dendritic cell and macrophage counterreceptors is required to prevent the clearance of donor lymphohematopoietic cells. *J. Exp. Med.* 194, 541–549
26 Chao, M.P. *et al.* (2011) Programmed cell removal: a new obstacle in the road to developing cancer. *Nat. Rev. Cancer* 12, 58–67
27 Linnartz, B. *et al.* (2012) Sialic acid on the neuronal glycocalyx prevents complement c1 binding and complement receptor-3-mediated removal by microglia. *J. Neurosci.* 32, 946–952
28 Meesmann, H.M. *et al.* (2010) Decrease of sialic acid residues as an eat-me signal on the surface of apoptotic lymphocytes. *J. Cell Sci.* 123, 3347–3356
29 Wang, Y. and Neumann, H. (2010) Alleviation of neurotoxicity by microglial human Siglec-11. *J. Neurosci.* 30, 3482–3488
30 Balasubramanian, K. and Schroit, A.J. (2003) Aminophosphospholipid asymmetry: a matter of life and death. *Annu. Rev. Physiol.* 65, 701–734
31 Lee, S.J. *et al.* (2011) Mechanism for phosphatidylserine-dependent erythrophagocytosis in mouse liver. *Blood* 117, 5215–5223
32 Albacker, L.A. *et al.* (2010) TIM-4, a receptor for phosphatidylserine, controls adaptive immunity by regulating the removal of antigen-specific T cells. *J. Immunol.* 185, 6839–6849
33 Dalli, J. *et al.* (2011) Annexin A1 regulates neutrophil clearance by macrophages in the mouse bone marrow. *FASEB J.* 26, 387–396
34 Hoeppner, D.J. *et al.* (2001) Engulfment genes cooperate with ced-3 to promote cell death in *Caenorhabditis elegans*. *Nature* 412, 202–206
35 Reddien, P. *et al.* (2001) Phagocytosis promotes programmed cell death in *C. elegans*. *Nature* 412, 198–202
36 Galvin, B.D. *et al.* (2008) *Caenorhabditis elegans* genes required for the engulfment of apoptotic corpses function in the cytotoxic cell deaths induced by mutations in lin-24 and lin-33. *Genetics* 179, 403–417
37 Darland-Ransom, M. *et al.* (2008) Role of *C. elegans* TAT-1 protein in maintaining plasma membrane phosphatidylserine asymmetry. *Science* 320, 528–531
38 Neukomm, L.J. *et al.* (2011) Loss of the RhoGAP SRGP-1 promotes the clearance of dead and injured cells in *Caenorhabditis elegans*. *Nat. Cell Biol.* 13, 79–86
39 Mergliano, J. and Minden, J.S. (2003) Caspase-independent cell engulfment mirrors cell death pattern in *Drosophila* embryos. *Development* 130, 5779–5789
40 Kuraishi, T. *et al.* (2009) Pretaporter, a *Drosophila* protein serving as a ligand for Draper in the phagocytosis of apoptotic cells. *EMBO J.* 28, 3868–3878
41 Li, W. and Baker, N.E. (2007) Engulfment is required for cell competition. *Cell* 129, 1215–1225
42 Olsson, M. and Oldenborg, P.A. (2008) CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcγ receptor-mediated but not scavenger receptor-mediated recognition by macrophages. *Blood* 112, 4259–4267
43 Oldenborg, P.A. *et al.* (2000) Role of CD47 as a marker of self on red blood cells. *Science* 288, 2051–2054
44 Khandelwal, S. *et al.* (2007) Reduced expression of CD47 during murine red blood cell (RBC) senescence and its role in RBC clearance from the circulation. *Transfusion* 47, 1725–1732
45 Lagasse, E. and Weissman, I.L. (1994) Bcl-2 inhibits apoptosis of neutrophils but not their engulfment by macrophages. *J. Exp. Med.* 179, 1047–1052
46 Park, Y.J. *et al.* (2008) PAI-1 inhibits neutrophil efferocytosis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11784–11789
47 Jaiswal, S. *et al.* (2009) CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 138, 271–285
48 Frasch, S.C. *et al.* (2008) NADPH oxidase-dependent generation of lysophosphatidylserine enhances clearance of activated and dying neutrophils via G2A. *J. Biol. Chem.* 283, 33736–33749
49 Maugeri, N. *et al.* (2009) Neutrophils phagocytise activated platelets in vivo: a phosphatidylserine, P-selectin, and β2 integrin-dependent cell clearance program. *Blood* 113, 5254–5265

50 Lindsten, T. *et al.* (2000) The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. *Mol. Cell* 6, 1389–1399
51 Zoller, E.E. *et al.* (2011) Hemophagocytosis causes a consumptive anemia of inflammation. *J. Exp. Med.* 208, 1203–1214
52 Overholtzer, M. *et al.* (2007) A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. *Cell* 131, 966–979
53 Lugini, L. *et al.* (2006) Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. *Cancer Res.* 66, 3629–3638
54 Wang, S. *et al.* (2009) Internalization of NK cells into tumour cells requires ezrin and leads to a programmed cell-in-cell death. *Cell Res.* 19, 1350–1362
55 Mormone, E. *et al.* (2006) Genotype-dependent priming to self- and xeno-cannibalism in heterozygous and homozygous lymphoblasts from patients with Huntington's disease. *J. Neurochem.* 98, 1090–1099
56 Neniskyte, U. *et al.* (2011) Neuronal death induced by nanomolar amyloid beta is mediated by primary phagocytosis of neurons by microglia. *J. Biol. Chem.* 286, 39904–39913
57 Fricker, M. *et al.* (2012) MFG-E8 mediates primary phagocytosis of viable neurons during neuroinflammation. *J. Neurosci.* 32, 2657–2666
58 McArthur, S. *et al.* (2010) Annexin A1: a central player in the anti-inflammatory and neuroprotective role of microglia. *J. Immunol.* 185, 6317–6328
59 Marin-Teva, J.L. *et al.* (2004) Microglia promote the death of developing Purkinje cells. *Neuron* 41, 535–547
60 Wakselman, S. *et al.* (2008) Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. *J. Neurosci.* 28, 8138–8143
61 Paolicelli, R.C. *et al.* (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458
62 Kao, A.W. *et al.* (2011) A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4441–4446
63 Neher, J.J. *et al.* (2012) Primary phagocytosis of neurons by inflamed microglia: potential roles in neurodegeneration. *Front. Pharmacol.* 3, 27
64 Kang, T.W. *et al.* (2011) Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 479, 547–551
65 Majeti, R. *et al.* (2009) CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 138, 286–299
66 Chao, M.P. *et al.* (2011) Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia. *Cancer Res.* 71, 1374–1384
67 Galluzzi, L. *et al.* (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* 19, 107–120
68 Yuan, J. and Kroemer, G. (2010) Alternative cell death mechanisms in development and beyond. *Genes Dev.* 24, 2592–2602
69 Borghans, J.A. and de Boer, R.J. (2007) Quantification of T-cell dynamics: from telomeres to DNA labeling. *Immunol. Rev.* 216, 35–47
70 Macallan, D.C. *et al.* (2005) B-cell kinetics in humans: rapid turnover of peripheral blood memory cells. *Blood* 105, 3633–3640
71 Hoath, S.B. and Leahy, D.G. (2003) The organization of human epidermis: functional epidermal units and phi proportionality. *J. Invest. Dermatol.* 121, 1440–1446
72 Lippens, S. *et al.* (2009) Cell death in the skin. *Apoptosis* 14, 549–569