Cell

Leading Edge
Essay

Decoding Cell Death Signals in Inflammation and Immunity

Laurence Zitvogel,[1,2,3,*] Oliver Kepp,[2,3,4] and Guido Kroemer[2,3,4,*]
[1]INSERM, U805
[2]Institut Gustave Roussy
[3]Université Paris-Sud
[4]INSERM, U848
F-94805 Villejuif, France
*Correspondence: zitvogel@igr.fr (L.Z.), kroemer@orange.fr (G.K.)
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Dying cells release and expose at their surface molecules that signal to the immune system. We speculate that combinations of these molecules determine the route by which dying cells are engulfed and the nature of the immune response that their death elicits.

Introduction

In a cycle that repeats several billions of times per day in the healthy human adult, dead cells attract scavengers—either neighboring cells or professional phagocytes (mostly macrophages)—that mediate their engulfment and digestion without leaving any trace, neither corpses nor graveyards. This interaction between dying cells and phagocytes reflects the baseline contribution of inflammation to normal tissue homeostasis (Metchnikoff and Ehrlich, 1990). Perturbations of this equilibrium due to the inappropriate death of noninflammatory cells or insufficient clearance of dying or dead cells by phagocytes can lead to autoimmune disease, as well as to pathological inflammation. Once inflammation is manifest or an immune response is mounted, their resolution or decline, respectively, requires the apoptosis and clearance of effector cells (Medzhitov, 2008). Therefore, understanding the pathways for cell death and clearance is instrumental for the exploration and therapeutic manipulation of inflammation.

When cells die in response to microbial infection, the local presence of pathogen-associated molecular patterns (PAMPs) triggers the innate (and eventually the cognate) immune response, marking the distinction between innocuous cell death (without PAMPs), which should be handled without an inflammatory response, and pathological cell death (with PAMPs), which should induce a response. In this Essay, we concentrate on cell death occurring in the absence of PAMPs and its impact on inflammation caused in the absence of infection. The generally accepted paradigm is that apoptosis, the physiological form of cell death, occurs without (and sometimes even with the active sequestration of) danger-associated molecular patterns (DAMPs). Apoptotic corpses can suppress the transcription of proinflammatory cytokine genes, promote the secretion of anti-inflammatory cytokines by phagocytes, and cause antigen-presenting cells to present dead-cell-antigen in a manner that promotes immunological tolerance. In contrast, necrosis, which often results from nonphysiological damage, leads to the exposure of DAMPs and consequent activation of inflammatory and immune effectors because DAMPs act on the same pattern recognition receptors as PAMPs (Kono and Rock, 2008). Nevertheless, the appealing notion that accidental necrosis would always elicit inflammation and potent immune responses whereas programmed apoptosis would be anti-inflammatory and tolerogenic is an oversimplification. For instance, this concept is challenged by the fact that in some cases, antigen from apoptotic cells triggers efficient immune responses (Green et al., 2009) and that necrosis can be executed in a programmed, highly regulated fashion (Garg et al., 2009).

Here, we explore the notion that it is the context in which cell death occurs that determines its impact on the inflammatory and immune response. We propose that particular combinations of cell death-associated molecules released from or exposed at the surface of dying or dead cells act like a combinatorial code to unlock distinct inflammatory and immune responses.

Cell Death-Associated Molecules

Dying or dead cells expose or release numerous molecules to attract inflammatory effectors ("find-me" signals) and to foster their engulfment ("eat-me" signals) so that the release of potential autoantigens is avoided. Here we enumerate some of the common characteristics of cell death-associated molecules (Table S1 available online).

"Find-Me" Signals for Chemotaxis

As cells die, they can release several factors that attract professional phagocytes, in particular macrophages. Among the most important find-me signals are nucleotides (such as ATP and UTP), which are released either through an active exocytosis-like process before the plasma membrane becomes permeable or passively when cells lose their integrity (Ghiringhelli et al., 2009). Through its action on cell surface purinergic receptors, in particular $P2Y_2$, released ATP attracts macrophages (Elliott et al., 2009). ATP also activates the NLRP3 inflammasome through its action on $P2RX_7$, thus stimulating the production of interleukin 1β (IL-1β) by macrophages or dendritic cells (Ghiringhelli et al., 2009). Apoptotic cells release lipid mediators such as lysophosphatidylcholine and sphingosine-1-phosphate (which presumably can be released before the plasma membrane breaks down). These lipids

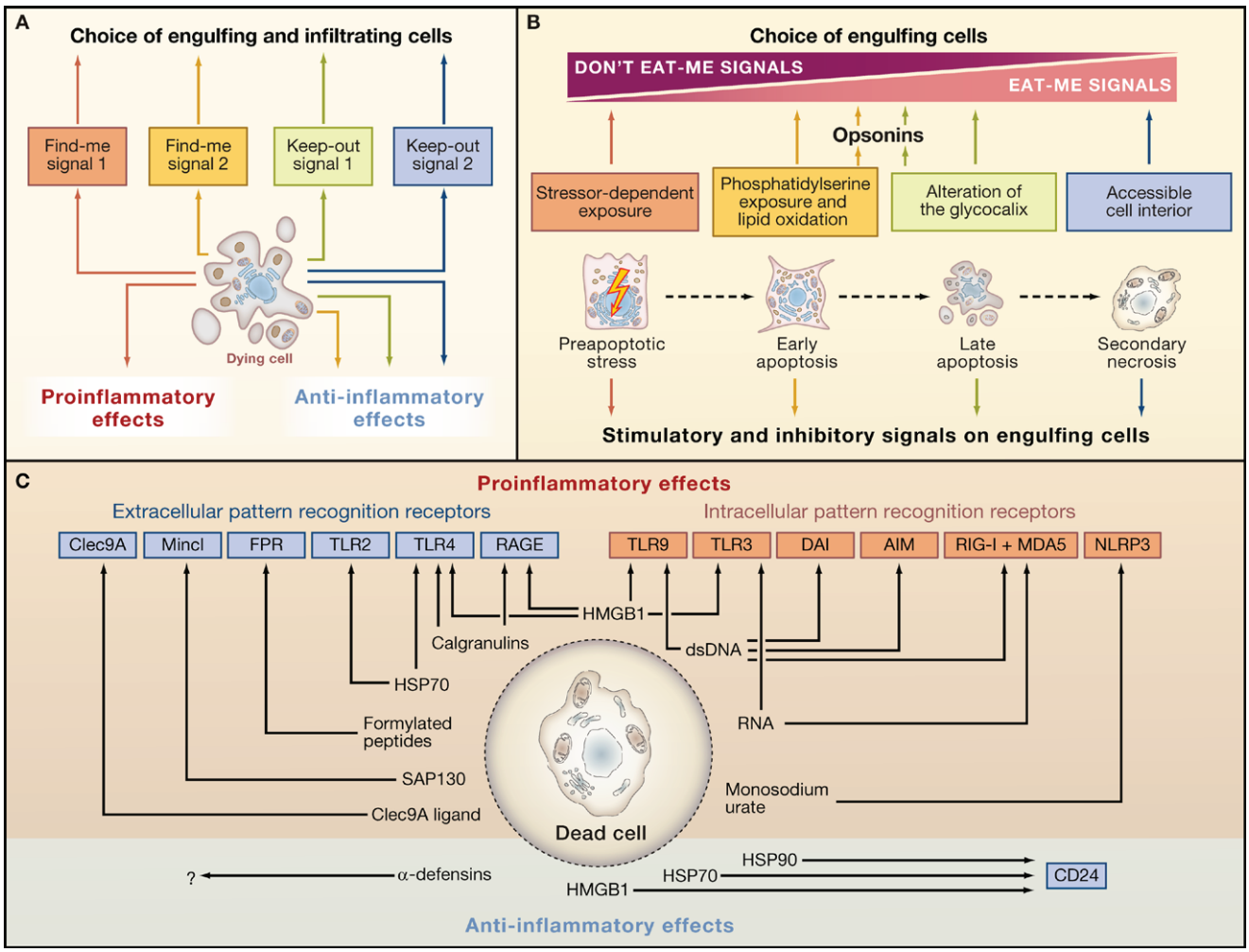

Figure 1. Cell Death-Associated Molecules

(A) Find-me signals. Chemotactic and chemotropic ("find-me") signals cause mobile phagocytic cells to migrate as a whole (chemotaxis) or extended part of the cell (chemotropism) toward the dying cell. Distinct find-me signals are generated during apoptosis and secondary necrosis and might attract different phagocytes. "Keep-out" signals repel defined inflammatory cells. Some find-me signals have anti-inflammatory effects, while others have proinflammatory potential.

(B) Eat-me signals. The exposure of engulfment ("eat-me") signals, combined with the downregulation of repulsion ("don't eat-me") signals, facilitates the engulfment of dying cells. Some eat-me signals (such as calreticulin) can be exposed before the most conserved signal, phosphatidylserine (PS), exposure occurs. Others manifest later, for instance when the glycocalix changes. A number of eat-me signals require opsonins as molecular bridges to phagocytes. Some receptors that perceive eat-me signals transmit anti-inflammatory signals.

(C) Hidden molecules. Primary or secondary necrosis causes the release of multiple molecules that are usually secluded ("hidden") within the intact plasma membrane and that can be perceived by pattern recognition receptors once they become accessible or are released. Some hidden molecules can mediate predominantly immunosuppressive effects. Moreover, phagocytes express TAM receptors that, upon ligation by HMGB1, HSP70, or HSP90, have anti-inflammatory effects.

attract macrophages and simultaneously may inhibit the release of proinflammatory cytokines (HMGB1, TNF-α, IL-12) and enhance the liberation of immunosuppressive factors (IL-10, PGE_2) from macrophages (Munoz et al., 2009). Cleaved proteins that result from the action of caspases (such as endothelial monocyte-activating polypeptide II) may also serve as chemoattractants at a later stage of cell death when they are released through the permeabilized plasma membrane (Munoz et al., 2009) and hence act as a backup signal when apoptotic cells have not been removed before they undergo secondary necrosis. Lactoferrin secreted by apoptotic cells serves as a "keep-out" signal to specifically suppress the potentially harmful recruitment of neutrophil granulocytes (Bournazou et al., 2009). Altogether, it appears that dying cells can emit a number of redundant signals that facilitate the chemotactic recruitment of specific phagocytes (Figure 1A).

"Eat-Me" Signals for Engulfment

The physicochemical properties of cell surfaces change as cells die and are engulfed by neighboring cells (for instance by epithelial cells or fibroblasts), macrophages, or immature dendritic cells. This process is facilitated by serum-derived proteins, known as opsonins, including growth arrest-specific gene 6 (Gas6), milk fat globule EGF/factor VIII (MFG-E8), β2-glycoprotein 1 (β2GP1), and annexin V. These proteins

bind to surface-exposed phosphatidylserine residues. Phosphatidylserine is usually only present in the inner leaflet of the plasma membrane. However, the death-associated increase in entropy, aided by the inactivation of specific lipid transferases, culminates in the surface exposure of phosphatidylserine, which together with other lipids, can become oxidized. The exposure of phosphatidylserine is efficiently induced upon caspase activation, as well as in a slower, caspase-independent fashion, in developmental and homeostatic cell death (Table S1). Several receptors on phagocytes assure the engulfment of cells exposing phosphatidylserine, including T cell immunoglobulin domain and mucin domain protein 4 (TIM4), the scavenger receptors CD36 and steroid receptor activator 1 (which both bind oxidized lipids), and the TAM family of receptors (which bind Gas6) comprised by Tyro2, Axl, and Mer. These latter receptors can suppress Toll-like receptor (TLR) signaling (Lemke and Rothlin, 2008), revealing one mechanism by which apoptotic cells suppress proinflammatory signals.

Before phosphatidylserine is exposed, cells can specifically translocate calreticulin from the endoplasmic reticulum (ER) lumen to the cell surface (Panaretakis et al., 2009). When calreticulin is surface exposed before phosphatidylserine, it may facilitate the engulfment of dying cells by immature dendritic cells, thereby increasing the immunogenicity of cell death (Obeid et al., 2007). After phosphatidylserine is exposed, in late stages of apoptosis, the cell surface glycosylation pattern changes, correlating with the recruitment of membranes from cytoplasmic organelles, in particular the ER, to the cell surface. This change in the glycocalix facilitates the binding of the opsonins, complement factor C1q, C-reactive protein (CRP), the long penetraxin (PTX-3), and the collectins (mannan-binding protein [MBL], surfactant proteins A and D [SP-A and -D]). The alteration of the glycosylation pattern of late apoptotic cells may serve as a back-up eat-me signal (Schulze et al., 2008). If find-me and distinct eat-me signals fail and apoptotic cells proceed to undergo secondary necrosis and lose the integrity of their plasma membrane, they can no longer form a synapse-like structure with macrophages required for the phagocytosis of the entire corpse. Reportedly, necrotic cells are engulfed through a macropinocytotic-like mechanism (Garg et al., 2009), suggesting that the cell death modality determines how dead cells are degraded and antigens contained in them are presented.

The clearance of stressed and dying cells can also be facilitated by the downregulation of "don't eat-me" signals (such as CD31 and CD47) that usually assure the repulsion of phagocytes (Table S1). Thus, there is a whole repertoire of eat-me and don't eat-me signals that are exposed in a cell death subroutine-dependent (and cell type-specific) fashion and that act on a variety of engulfment-promoting and -inhibitory receptors, respectively. Some of these receptors are expressed on specific subsets of engulfing cells, suggesting a combinatorial interplay of receptor-ligand interactions in which the dying cell "chooses" the engulfing cell in its vicinity (Figure 1B).

"Hidden Molecules" as Inflammatory Signals

In developmental or homeostatic cell death, the agonizing cells are efficiently cleared before their plasma membranes become permeabilized and molecules that are usually inaccessible ("hidden") are released or exposed. For instance, a yet unknown preformed molecule that remains associated with necrotic cells (and hence is likely a part of the insoluble cytoskeleton) serves as a ligand for the SYK-coupled C-type lectin receptor Clec9a. Clec9a is expressed on CD8α^+ dendritic cells that stimulate the cross-presentation of antigens associated with dead cells (Sancho et al., 2009).

Necrotic cells release several alarmins, which are soluble proteins with proinflammatory properties. SAP130 is a spliceosome component that is liberated from necrotic cells and activates the C-type lectin receptor Mincle, which stimulates the recruitment of neutrophils to the site of cell death (Yamasaki et al., 2008). Necrotic cells also release heat shock proteins (such as HSP70, HSP90, and gp96), in particular when they have been previously upregulated in response to stress. These then stimulate the pattern recognition receptors TLR2 and TLR4. Calgranulins comprise three proteins, S100A8 (calgranulin A), S100A9 (calgranulin B), and S100A12 (calgranulin C), that are predominantly released by necrotic neutrophils, monocytes, and activated macrophages, respectively, and stimulate TLR4 or RAGE (receptor for advanced glycation end-products). IL-1α can be passively released from necrotic cells and stimulate inflammation. N-formylated mitochondrial peptides synergize with mitochondrial transcription factor A (TFAM), the mitochondrial homolog of HMGB1, to induce IL-8 release from monocytes (Table S1). High-mobility group box 1 (HMGB1) protein is a nuclear protein that is released via the cytoplasm into the microenvironment of dying cells when their plasma membrane ruptures. HMGB1 release is often more efficient when it occurs in primary necrosis as opposed to secondary necrosis (that is, necrosis after apoptosis) (Bianchi, 2009). Moreover, apoptosis-associated redox reactions can oxidize and inactivate HMGB1 (Kazama et al., 2008). These reports imply that, in "normal" apoptosis, HMGB1 is retained in the nucleus or released in an inactive form when the cells switch to secondary necrosis. This contrasts with the observation that HMGB1 released from anthracylin-treated cancer cells can activate TLR4 (Apetoh et al., 2007).

Depending on the molecules that it binds to, HMGB1 preferentially interacts with different pattern recognition receptors. HMGB1 can form highly inflammatory complexes with single-stranded DNA, lipopolysaccharide, IL-1β, and nucleosomes, which interact with TLR9, TLR4, IL-1R, and TLR2 receptors, respectively. In addition, uncomplexed HMGB1 can interact with RAGE (Bianchi, 2009) and TLR4 (Apetoh et al., 2007). As a result, extracellular HMGB1 activates macrophages and dendritic cells and promotes neutrophil recruitment. It also plays a major role in septic shock, an extreme systemic inflammation in which massive cell death correlates with an increase in serum HMGB1 levels (Bianchi, 2009).

Necrotic cells also release RNA (which stimulates TLR3) and genomic double-stranded DNA (dsDNA). Ectopic, extranuclear dsDNA stimulates TLR9 and other pattern recognition receptors including RIG-I and MDA5 for the acti-

vation of IRF3 and NF-κB (leading to the production of IFN-β and CXCL10), as well as the AIM2 inflammasome (which facilitates the secretion of IL-1β) (Kawai and Akira, 2009). Thus, dsDNA from dying cells that have not been correctly disposed of can elicit multiple redundant alarm signals and must be degraded to avoid pathogenic inflammation. Indeed, deficiency for the extracellular DNase I causes a lupus-like syndrome in mice, and DNase I mutations in humans are associated with lupus (Martinez Valle et al., 2008). Deficiencies in the intracellular DNase II also cause polyarthritis in mice (Nagata et al., 2010). It is currently unknown whether so-called "extracellular traps," which are produced by dying neutrophils or mast cells and consist of a chromatin-DNA backbone with attached antimicrobial peptides and enzymes that trap and kill microbes (Wartha and Henriques-Normark, 2008), activate pattern recognition receptors or whether their particular architecture precludes such a process. Other factors released from necrotic cells include monosodium urate microcrystals that form when uric acid (soluble within cells) precipitates in the sodium-rich extracellular fluid. Monosodium urate crystals stimulate the inflammasome of macrophages and dendritic cells (Martinon et al., 2009) and may contribute as an endogenous adjuvant to increase the immunogenicity of necrotic cells (Kono and Rock, 2008).

It would be an oversimplification, though, to postulate that all hidden molecules are proinflammatory in nature. For instance, human neutrophils contain high concentrations of the four human neutrophil peptides (HNP) 1–4, a series of α-defensins that are stored in the azurophilic granules and are released upon apoptotic or necrotic cell death to inhibit the secretion of inflammatory cytokines and nitric oxide from macrophages (Miles et al., 2009). Moreover, HMGB1 (as well as HSP70 and HSP90) can engage inhibitory receptors such as CD24 that dampen their proinflammatory effects (Chen et al., 2009). This implies that, depending on the specific context, hidden molecules can trigger both stimulatory and regulatory receptors that trigger and limit inflammation, respectively (Figure 1C).

Stress before Death

Different stressors elicit a limited pattern of apparently homogeneous lethal morphotypes, mostly apoptosis and necrosis. However, cellular stress responses—which precede cell death—are highly diversified, meaning that the history of the preapoptotic events conditions the internal composition and even the surface characteristics of cellular corpses. Moreover, the apoptotic and necrotic execution phase itself can involve the variable contribution of distinct catabolic hydrolases including caspases and caspase-independent death effectors, implying that similar morphologies may have been acquired through distinct biochemical routes, thereby influencing the exposure and release of cell death-associated molecules.

Heat Shock Proteins

The transcriptional upregulation of heat shock proteins (HSPs) is part of the general response to cellular stress. Certain inducible HSPs such as HSP70 and HSP90 can translocate to the plasma membrane (HSP70 through binding to phosphatidylserine and the sphingolipid Gb3) and then serve as danger signals. HSPs may facilitate the interaction with surface receptors of antigen-presenting cells (such as CD91, LOX1, CD40) and reportedly mediate the transfer of antigenic peptides from the stressed cell to the antigen-presenting cell (Table S1). HSPs stimulate TLR4, and HSP70 reportedly stimulates dendritic cell maturation by upregulating CD40 and CD86. Moreover, the anticancer agent bortezomib (a proteasome inhibitor) induces the expression of HSP90 on the surface of dying human myeloma tumor cells, facilitating their recognition by dendritic cells and the generation of antitumor T cells (Spisek et al., 2007). These examples illustrate how a stress response can increase the proinflammatory and immunogenic properties of agonizing cells.

ER Stress Response

The ER stress has been involved in the lipotoxic death of macrophages, for instance in morbid obesity and within atherosclerotic lesions. Alleviation of ER stress by a chemical chaperone or knockout of the fatty acid-binding protein-4 (aP2), which is specific to antigen-presenting cells, prevents lipotoxic macrophage death and atherosclerosis (Erbay et al., 2009). In response to some cell death inducers including ionizing irradiation and chemotherapeutic agents (such as anthracyclins or oxaliplatin), cells can mount an ER stress response that culminates in the phosphorylation of eIF2α (eukaryotic initiation factor 2α) by the kinase PERK and the later caspase-8-mediated cleavage of the ER protein BAP31, causing the anterograde traffic of calreticulin-containing vesicles from the ER to the Golgi apparatus and exocytosis-mediated calreticulin exposure (Panaretakis et al., 2009). Preapoptotic exposure of calreticulin is an important signal for immunogenic cell death (Obeid et al., 2007), perhaps because cells that expose calreticulin before they expose phosphatidylserine are preferentially targeted to immature dendritic cells rather than to macrophages. Multiple viruses can inhibit the calreticulin exposure pathway, perhaps as a strategy for the avoidance of immune responses.

Lysosomal Membrane Permeabilization and Inflammasome Activation

The terms pyroptosis and pyronecrosis have been introduced to describe a particular form of cell death in macrophages that is induced by bacterial infection, is accompanied by caspase-1 activation, and hence leads to the release of pyrogenic interleukins, in particular IL-1β, whose precursors must be cleaved by caspase-1 to be released (Kepp et al., 2010). Caspase-1 activation relies on the stimulation of the inflammasome. Activation of the inflammasome can be triggered by lysosomal membrane permeabilization (LMP), for instance in macrophages that phagocytose silica particles (the causative agent of silicosis), aluminum salt crystals (one of the most widely used adjuvants), or microglial cells that incorporate the fibrillar peptide amyloid-β (whose accumulation plays a major role in Alzheimer's disease). LMP, which is a frequent initiating event of cell death, culminates in the lysosomal release of cathepsin B, which activates the NLRP3 inflammasome and hence stimulates the production of proinflammatory IL-1β (Martinon et al., 2009). Intriguingly, caspase-1 activation is also involved in the unconventional secretion of multiple leaderless proteins such as pro-IL-1α, thioredoxin (important for inflammation), fibroblast growth factor

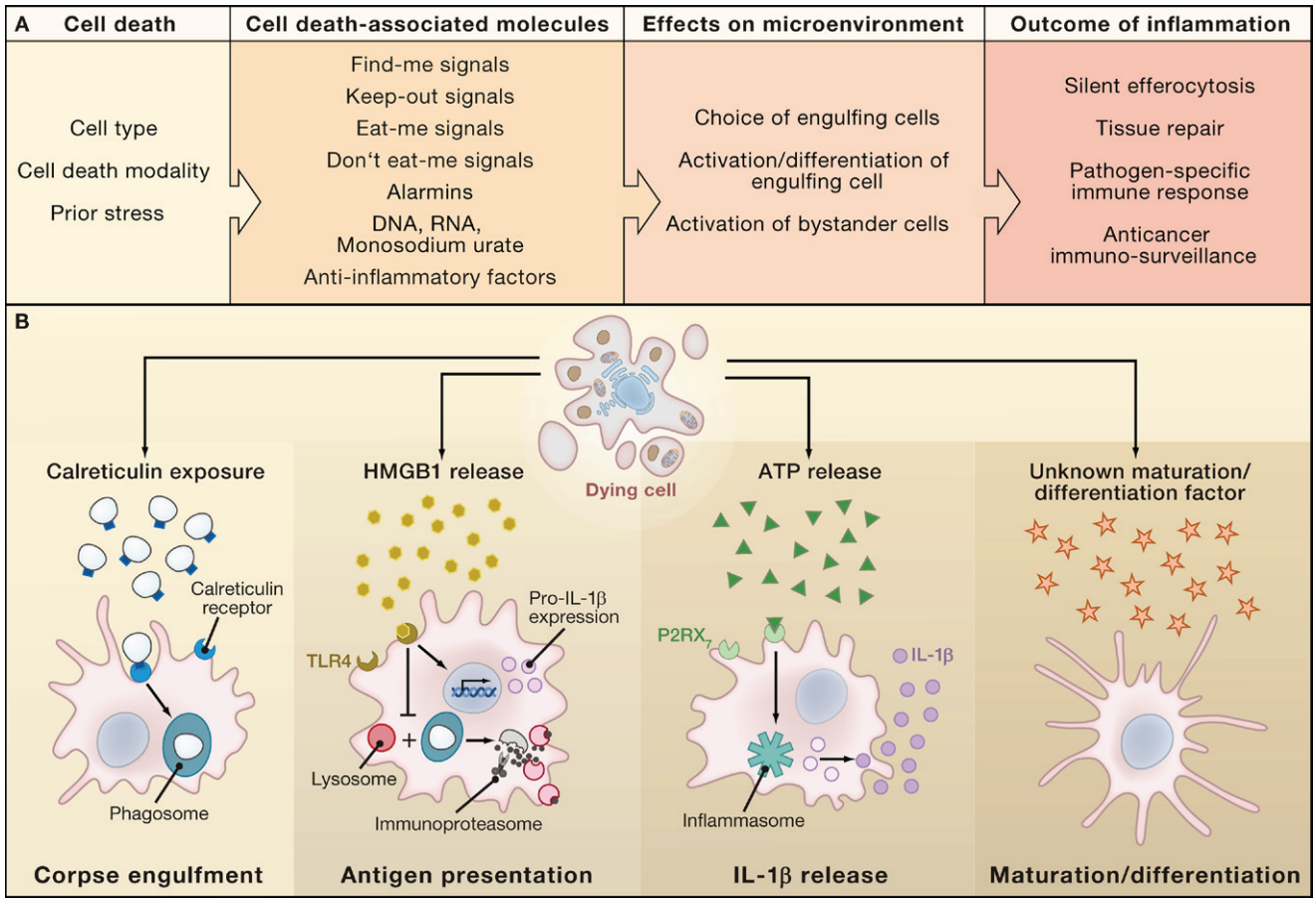

Figure 2. A Combinatorial Code Links Cell Death to the Outcome of Inflammation

(A) The path from cell death to inflammation. The peculiar characteristics of the dying cells determine the nature of the cell death-associated molecules that are exposed or released. These molecules mediate the effects of dying cells on the microenvironment, in particular the choice and the activation of the engulfing cells and possible effects on bystander cells, thus determining the outcome of inflammation.

(B) Peculiarities of immunogenic cell death. Dying cells expose calreticulin at an early stage of the apoptotic process, which facilitates engulfment by dendritic cells. HMGB1 released from dying cells binds to TLR4 on dendritic cells, thus favoring antigen cross-presentation and upregulating pro-interleukin-β (pro-IL-1β). ATP liberated from dying cells binds to the purinergic receptor $P2RX_7$ on dendritic cells, activates the NLRP3 inflammasome, and stimulates the liberation of IL-1β, which polarizes $CD8^+$ T cells toward interferon-γ production. A hypothetical dendritic cell maturation factor remains to be characterized.

(FGF)-2 (important for tissue repair and angiogenesis), and calreticulin (important for wound healing) (Keller et al., 2008), suggesting that cell death preceded by caspase-1 activation would be particularly efficient in stimulating inflammation and tissue repair.

DNA Stress Response

The DNA damage response (DDR) can be stimulated by ionizing irradiation, by genotoxic agents including some chemotherapeutic agents, as well as by oncogenic stress. Indeed, the unscheduled activation of oncogenes induces a DDR that ultimately leads to the activation of molecules (such as the kinases ATM and CHK2 and the tumor suppressor protein p53) that trigger apoptosis or senescence (see below) unless they are inactivated. This "intrinsic barrier" can obstruct oncogenesis and is backed up by an "extrinsic barrier" in which the DDR stimulates the surface expression of NKG2D ligands. NKG2D, a well-characterized stimulatory receptor that is expressed by natural killer (NK) cells and some T cells, recognizes such ligands, stimulating the lysis of the tumor cells and hence erecting part of the extrinsic barrier. The DDR also induces the expression of another NK cell receptor ligand, CD155, and that of death receptor 5 (DR5), a receptor of TRAIL (Raulet and Guerra, 2009). It remains to be determined in which specific circumstances the innate immune response is elicited by incipient tumors that activate (and eventually succumb to) the DDR as a result of oncogenic stress. Moreover, it remains elusive whether this innate reaction contributes to chronic inflammation (and hence stimulates neoplastic transformation) or rather facilitates a subsequent cognate anticancer immune response (and hence contributes to anticancer immunosurveillance).

Senescence

Senescence is a near-to-irreversible arrest of the cell cycle in the G_1 phase that can precede cell death. The conditional reactivation of p53 in hepatocellular carcinomas induces cellular senescence, followed by the elimination of tumor cells by innate immune effectors. Gadolinium chloride (a macrophage toxin), as well as neutralizing antibodies to suppress neutrophil or NK cell function, delayed

tumor regression following p53 reactivation (Xue et al., 2007), indicating that p53-induced senescence can stimulate an effective anticancer response that is mediated by innate immune effectors. Senescent cells can upregulate intercellular adhesion molecule 1 (ICAM1) as well as NKG2D ligands (Raulet and Guerra, 2009), but it is currently unknown whether this is the mechanism through which senescent cells are destroyed by innate immune effectors. Senescent cells also express a series of cytokines such as IL-6, IL-8, GROα, and TGF-β, which interact with their respective receptors in an autocrine fashion to maintain the cells in the senescent stage and might exert paracrine effects on inflammatory cells or innate immune effectors (Bartek et al., 2008). It is unclear whether this "senescence-associated secretory phenotype" (SASP) links cellular senescence to organismal aging (Franceschi et al., 2007). Moreover it is not known whether SASP stimulates tumor progression or rather contributes to the elimination of senescent (and potentially oncogenic) cells by innate immune effectors.

Autophagy Preceding Death

Macroautophagy (hereafter referred to as "autophagy") is frequently activated in response to cellular stress before cells die, including in developmental cell death. Autophagy is essential for the maintenance of intracellular ATP levels (and possibly for its release), the secretion of the find-me signal lysophosphatidylcholine, and the efficient exposure of the eat-me signal phosphatidylserine, implying that autophagy determines the kinetics of corpse removal (and perhaps the nature of the phagocyte) (Levine and Kroemer, 2008). Autophagy within dying antigen donor cells can improve the cross-presentation of tumor antigens or viral antigens by dendritic cells perhaps because autophagosomes ferry antigens to dendritic cells through an as yet unknown mechanism (Li et al., 2008) or because higher amounts of type I interferon are induced (Uhl et al., 2009). Autophagy may also influence the surface proteome of dying cells and stimulate the preapoptotic secretion of HMGB1 (Thorburn et al., 2009). In this context, it appears intriguing that many virus-encoded proteins inhibit the autophagic machinery (Orvedahl and Levine, 2009), a strategy that might subvert antiviral immune responses. Moreover, many oncogenes, as well as the inactivation of tumor suppressor genes, result in autophagy inhibition, especially in early oncogenesis (Levine and Kroemer, 2008), thus constituting a mechanism that might facilitate the escape of transformed cells from immunosurveillance. There are multiple intersections between autophagy and inflammation (Virgin and Levine, 2009). For example, the IKK complex, which mediates proinflammatory NF-κB activation, is also required for the induction of autophagy (Criollo et al., 2010). It remains unclear to what extent and through which mechanisms the increase in longevity mediated by the induction of autophagy at the whole-body level (by caloric restriction, rapamycin, resveratrol, or spermidine) (Morselli et al., 2009) is accompanied by a reduction of inflammation associated with aging.

A Combinatorial Code?

With regard to cell death, the teleological purpose of inflammation is to clear corpses, to stimulate the replacement of lost cells, to detect cell death induced by infectious agents, to alert the host defense, and possibly to strengthen the exogenous barrier against oncogenesis (Figure 2A). The preapoptotic phase of lethal pathways and frustrated attempts to cope with stress have a profound effect on the cell surface proteome. These factors may affect the cellular release of find-me signals, exposure of eat-me signals, disclosure of hidden molecules, and secretion of cytokines. Together, the release of positive and negative chemotactic signals and the ensemble of changing cell surface structures influence the choice of the engulfing cell, its activation, and subsequent differentiation.

Dying and engulfing cells interface, first by building a sort of intercellular synapse through a zipper-like mechanism (at least in the case of apoptosis), then by juxtaposing phagocytic cargo (from the engulfed cell) with endocytic pattern recognition receptors (such as the RNA- and DNA-sensing TLRs from the engulfing cells). This suggests that the engulfing cell can detect multiple properties of the dying or dead cell simultaneously. Indeed, it is essential that PAMPs (Blander and Medzhitov, 2006) or cell death-associated molecules (Obeid et al., 2007) are closely associated with dying cells so that they are taken up together by the same dendritic cell. If the PAMP or the cell death-associated molecule is present in the environment on unrelated cells, it fails to elicit efficient antigen presentation, underscoring the importance of signal context.

The simultaneous detection of multiple properties of dying and dead cells within the same compartment enables the primary inflammatory cell, the macrophage, or the immature dendritic cell to decrypt the information by sensing multiple cell death-associated molecules (and, if present, PAMPs) and to mount an appropriate response (Figure 2A). For example, the preapoptotic exposure of calreticulin, the apoptotic secretion of ATP, and the postapoptotic release of HMGB1 are all required for dying cells to stimulate the presentation of dead cell antigens by dendritic cells and the polarization of the T cell response toward the production of IFN-γ, which is essential for efficient antiviral and antitumor immune responses (Apetoh et al., 2007; Ghiringhelli et al., 2009; Obeid et al., 2007). Intriguingly, in this scenario the most abundant ER protein (calreticulin), one of the most abundant intracellular metabolites (ATP), and the most abundant nonhistone chromatin-binding proteins (HMGB1) act in an ectopic location to compose a spatiotemporal code that translates cell death into a cognate immune response (Figure 2B).

We speculate that this code regulates the relationship between dying cells and their microenvironment. A combinatorial code would unite several cell death-associated molecules in a spatiotemporary sequence that—within the context of signals originating from surrounding cells—then unleashes the silent clearance of dead cells, distinct tissue repair responses, recruitment of additional inflammatory effectors, or immune reactions (Figure 2A). In this view genetic deficiencies or acquired defects that perturb the appropriate interpretation of this combinatorial code would give rise to major perturbations in tissue homeostasis leading to insufficient, excessive, or maladaptive inflammatory and immune reactions (Table S2). Resolving the many remaining mysteries of this code constitutes the challenge for future investigation.

Supplemental Information

Supplemental information includes two tables and can be found with this article online at doi:10.1016/j.cell.2010.02.015.

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