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PERSPECTIVES

OPINION

Regulated cell death and inflammation: an auto-amplification loop causes organ failure

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Abstract | Regulated cell death (RCD) is either immunologically silent or immunogenic. RCD in parenchymal cells may lead to the release of damage-associated molecular patterns that drive both tissue inflammation and the activation of further pathways of RCD. Following an initial event of regulated necrosis, RCD and inflammation can induce each other and drive a local auto-amplification loop that leads to exaggerated cell death and inflammation. In this Opinion article, we propose that such crosstalk between pro-inflammatory and RCD pathways has pathophysiological relevance in solid organ failure, transplantation and cancer. In our opinion, clinicians should not only prescribe immunosuppressive treatments to disrupt this circuit, but also implement the neglected therapeutic option of adding compounds that interfere with RCD.

Tissue injury and inflammation are tightly linked processes and both are associated with the release of damage-associated molecular patterns (DAMPs) from dying cells[1]. Cell death can occur through unregulated necrosis — such as that which occurs in trauma — and also as a result of various forms of regulated cell death (RCD) (BOX 1). In this Opinion article, we suggest that these pathways represent neglected therapeutic targets for the prevention of inflammation because they trigger a 'necro-inflammatory' environment, which may cause organ failure and shock.

Regulated cell death pathways

RCD pathways can be categorized in different ways, including on the basis of whether or not they are caspase dependent[2], and whether or not they are immunogenic. The Nomenclature Committee on Cell Death has referred to a plethora of pathways in an attempt to include all published data that are available[3]. The pathways of RCD that we consider to be crucial are summarized in FIG. 1. However, this does not exclude the possibility that other pathways of RCD might also be of relevance.

Caspase-dependent forms of cell death. Apoptosis and pyroptosis are caspase-dependent forms of RCD. Apoptosis does not lead to plasma membrane rupture and therefore is the least immunogenic mode of cell death, although it is not entirely immunologically silent[4]. Caspase 8 is essential for death receptor-mediated apoptosis in mice, but caspase 8 preferentially heterodimerizes with the caspase-like protein FLICE-like inhibitory protein (FLIP; also known as CFLAR) to proteolytically inactivate a spontaneously occurring immunogenic cell death process, known as necroptosis, by inactivating receptor-interacting serine/threonine-protein kinases (RIPKs)[5,6]. Caspase 8 also has an additional anti-inflammatory effect during apoptosis by preventing RIPK3-dependent inflammasome activation[7–10].

Pyroptosis also depends on caspases, and may elicit an apoptotic[11] or necrotic morphology in cells[12,13]. In contrast to apoptosis, pyroptosis does not rely on caspase 3, caspase 7, caspase 8 or caspase 9, but instead depends on the activation of caspase 1 and caspase 11, which are involved in cytokine maturation and necrotic cell death, respectively[14]. This renders pyroptosis a highly immunogenic form of cell death. Pyroptosis may be regulated by the plasma membrane channel short transient receptor potential channel 1 (TRPC1)[15] and by guanylate-binding proteins[16], and the *in vivo* relevance of pyroptosis has been demonstrated in diverse models of bacterial[17] and viral[18,19] infection.

Caspase-independent forms of cell death. A number of caspase-independent forms of RCD have been described. These are necroptosis, mitochondrial permeability transition-mediated regulated necrosis (MPT-RN), ferroptosis and NET release-associated cell death (which is known as NETosis) (FIG. 1).

Necroptosis requires RIPK3-mediated[20–22] phosphorylation of mixed lineage kinase domain-like protein (MLKL)[23,24], which is an intracellular pseudokinase[25,26] that causes plasma membrane rupture through its amino-terminal bundle–brace motif[27,28]. Necroptosis may be activated by: signalling from death receptors (namely, tumour necrosis factor receptor (TNFR), TNF-related apoptosis-inducing ligand receptor (TRAILR; also known as TNFRSF10) or FAS (also known as CD95 and TNFRSF6)) through RIPK1; by receptors that sense viral nucleotides via the molecule DNA-dependent activator of interferon (IFN)-regulatory factors (DAI; also known as ZBP1); by Toll-like receptors (TLRs) via the adaptor molecule TIR domain-containing adaptor protein inducing IFNβ (TRIF; also known as TICAM1); and by IFNs through the activation of RIPK1 by protein kinase R (also known as EIF2AK2)[14,29]. As recently reviewed in detail[29], necroptosis has been detected in mouse models of disease — including viral and bacterial infections, severe inflammatory response syndrome, neuronal disorders, ischaemia–reperfusion injury, and diseases affecting the eye or the gastrointestinal tract and visceral organs — and has recently been directly confirmed in human disorders[29,30]. Necroptosis may be blocked therapeutically using necrostatins[31–33], which are compounds that maintain RIPK1 in an inhibitory conformation that prevents RIPK1-mediated necroptosis[49,105–108].

Box 1 | **Regulated cell death: forms and manifestations**

Cells that are exposed to extreme stimuli (for example, high temperatures and steep osmotic gradients) disassemble, hence dying in an 'accidental' manner. Conversely, cell death that is elicited by most pathophysiological conditions is 'active' or 'regulated', implying that it relies on certain molecular machinery and can be genetically or pharmacologically manipulated. For decades, forms of cell death has been defined as 'apoptotic', 'autophagic' or 'necrotic' on the basis of their morphological manifestations, an approach that is today deemed inappropriate. Rather, the biochemical events that accompany cell death are currently used to identify its nature. Thus, apoptosis is defined as a caspase-dependent type of cell death that differs from pyroptosis, which also involves caspases, in that the latter is accompanied by the release of interleukin-1β (IL-1β) and IL-18 from dying cells. Necroptosis is dependent on receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL) but not on mitochondria[48], whereas mitochondrial permeability transition (MPT)-mediated regulated necrosis (MPT-RN) crucially depends on cyclophilin D. The demise of neutrophils is accompanied by the rapid release of so-called neutrophil extracellular traps (NETs) — which are DNA-containing structures with antibacterial activity — and relies on reactive oxygen species, and is therefore referred to as NETosis. Glutathione peroxidase 4 (GPX4)-sensitive ferroptosis and poly(ADP-ribose) polymerase 1 (PARP1)-dependent parthanatos are additional pathways of regulated cell death (RCD). The potential overlap between RCD pathways is a matter of debate and investigation.

Importantly, the inhibition of caspases does not avoid cellular demise, but in some settings merely delays it or changes its morphological and biochemical manifestations. This may indicate that caspases do not cause apoptosis, but simply accelerate it or constitute one of its biochemical manifestations. It is therefore tempting to speculate that the event that ultimately and causally underlies all forms of RCD may be the same, perhaps a bioenergetic crisis involving the loss of ATP-producing capacity and reducing equivalents. In this entirely speculative scenario, apoptosis, necroptosis and MPT-RN would differ from each other only in terms of apical signalling and manifestations. The experimental evaluation of this hypothesis is awaited.

Mitochondrial permeability transition (MPT) has been noted to contribute to RCD in both apoptosis[34] and regulated necrosis (MPT-RN)[35–37]. The MPT pore (MPTP) is controlled by the mitochondrial matrix protein cyclophilin D (also known as PPID)[35,38]. MPT-RN contributes to ischaemia–reperfusion injury and other types of acute organ failure[14]. Compounds such as sanglifehrin A and cyclosporine A prevent MPT *in vitro* and MPT-RN *in vivo*, independently of their calcineurin-mediated immunosuppressive properties[39].

The hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1) triggers a form of regulated necrosis, termed parthanatos, in several disease models[14]. Many studies have examined the effects of PARP1 in the setting of cancer, where PARP1 inhibitors are thought to increase cancer cell death by blocking the DNA repair functions of PARP1 (REF. 2). However, it remains possible that the tumoricidal effects of PARP1 inhibitors may also be owing to their prevention of hypoxia-induced RCD in the necrotic core of the tumour.

Ferroptosis is an iron-dependent pathway of RCD that was first identified when a compound library was screened for selective lethality in RAS-transformed tumour cells[40–42]. The small molecule erastin was found to induce ferroptosis through the inhibition of system X_C^-, a plasma membrane cystine/glutamate antiporter that supplies tumour cells with cystine (the oxidized form of cysteine) which is required for glutathione biosynthesis[43]. Upon depletion of intracellular glutathione, glutathione peroxidase 4 (GPX4) no longer repairs accumulating lipid peroxides, resulting in lipid peroxidation and cell death[44,45].

NETs made of DNA and are rapidly released from neutrophils in a series of events that involves coordinated nuclear breakdown and chromatin decondensation. This event was initially described as a means of host defence against circulating bacteria[46]. NETosis is important for host defence against *Staphylococcus aureus* sepsis[47]. Intact anuclear neutrophils have been detected in human abscesses, suggesting that these cells have released NETs[47].

Importantly, further investigation is required to establish whether these and other proposed pathways of cell death are truly distinct or not[14]. In this sense, it is likely that there is overlap between MPT-RN and parthanatos, whereas necroptosis and ferroptosis have been clearly separated[43,48].

Auto-amplification of organ injury

Regulated cell death triggers inflammation. Cytoplasmic constituents include a wide range of DAMPs that bind pattern recognition receptors (PRRs; see Supplementary information S1 (table)). Some DAMPs seem to be specifically released as a result of certain types of RCD — for example, the cytokine interleukin-33 (IL-33) is thought to be specifically released by necroptotic cells[49]. Given the dynamics of plasma membrane rupture during necrosis, which certainly occurs in ischaemia–reperfusion injury[50], we intuitively tend to interpret necrosis as a strong pro-inflammatory trigger, but what is the experimental evidence for this hypothesis?

In mice with an intestinal epithelial cell-restricted deletion of the gene encoding FAS-associated death domain protein (FADD)[51] or caspase 8 (REF. 52), a spontaneous inflammatory bowel disease develops, which is rescued by backcrossing the animals onto a RIPK3-deficient background. These results suggest that this disease may be a necroptosis-driven systemic disorder. In line with these findings, mice with a functionally deficient mutant version of RIPK1 are completely protected from hyperacute shock induced by TNF and the pan-caspase inhibitor zVAD[53], which is another model that is partially reversed on a RIPK3-deficient background[54,55]. Similarly, chronic proliferative dermatitis (*cpdm*) mice, which are deficient in SHARPIN[56], develop a spontaneous necrotizing dermatitis that is partially reversed on a RIPK3-deficient background[57], and completely reversed on a RIPK1 kinase-dead[53] or TNFR1-deficient background[57]. Several other lines of evidence originate from ischaemia–reperfusion injury studies in which the inhibition of necroptosis, MPT-RN or parthanatos has been associated with lower serum concentrations of pro-inflammatory cytokines and less immune cell infiltration[39,58–65].

Apart from the activation of PRRs, one possible route by which the release of DAMPs from necrotic cells might promote pro-inflammatory signalling in bystander cells is through heat-induced necrosis, which has been demonstrated to activate AKT[66]. The control of RCD by phosphoinositide 3-kinase (PI3K)–AKT signalling and the role of this pathway as a potential therapeutic target have been recognized for over a decade[67]. It has also been suggested that AKT activation is induced by necrotic but not apoptotic cell death[66]. The activation of AKT was shown to correlate with poor outcomes in ischaemia–reperfusion injury and sepsis[68], and in sepsis-associated acute lung injury[69]; however, this speculative association does not provide strong evidence for a causative role of AKT in systemic inflammation. In this sense, it is reasonable to assign apoptosis a role in extinguishing the pro-inflammatory properties of dying cells, as has been previously discussed by others[70].

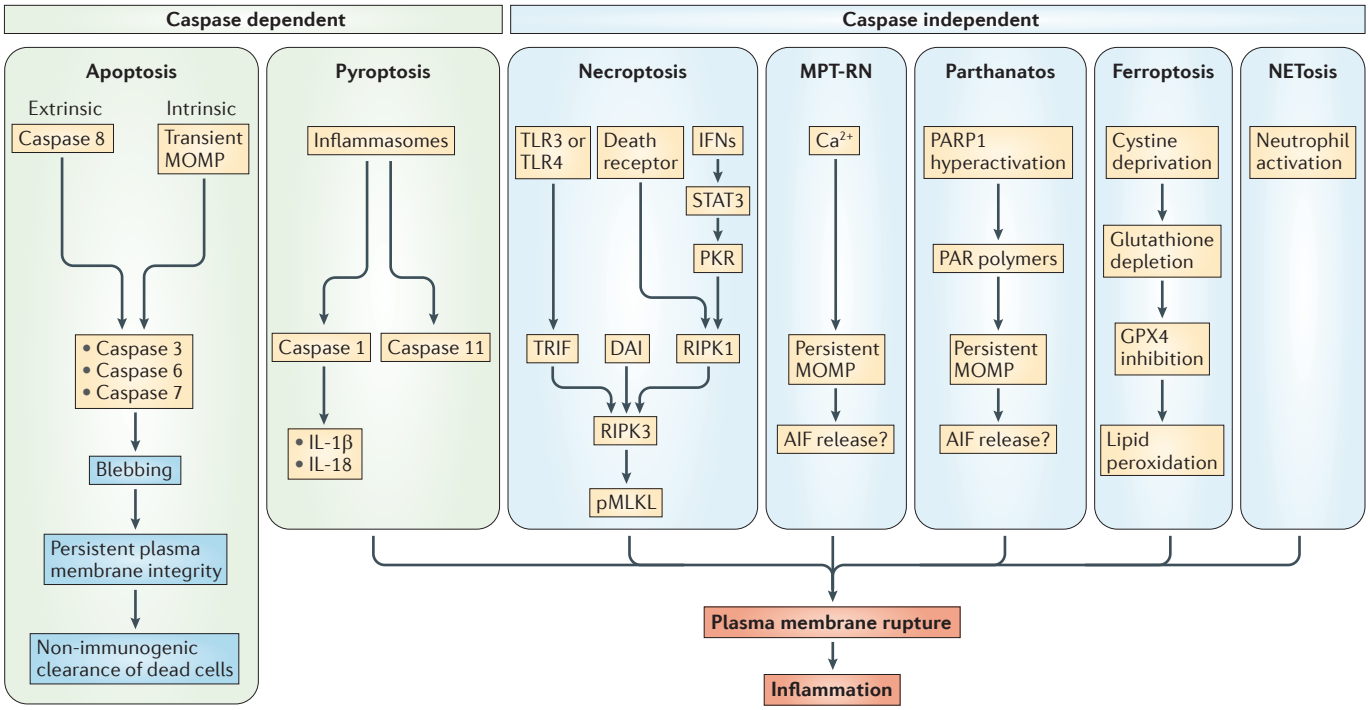

Figure 1 | **Signalling pathways of regulated cell death.** Regulated cell death (RCD) pathways may be classified by whether or not they are caspase dependent. Classical apoptosis is caspase dependent and is associated with long-lasting plasma membrane integrity. As such, it represents the least immunogenic form of cell death. Apoptosis pathways can be described as extrinsic or intrinsic, depending on whether or not they involve mitochondrial signalling. Necroptosis, mitochondrial permeability transition-mediated regulated necrosis (MPT-RN), ferroptosis and NETosis are caspase-independent forms of RCD and are highly immunogenic. Pyroptosis is caspase dependent, but is also a highly immunogenic type of cell death. AIF, apoptosis-inducing factor; DAI, DNA-dependent activator of IFN-regulatory factors; GPX4, glutathione peroxidase 4; IFN, interferon; IL, interleukin; MOMP, mitochondrial outer membrane permeabilization; NET, neutrophil extracellular trap; PAR, poly(ADP-ribose); PARP1, PAR polymerase 1; PKR, protein kinase R; pMLKL, phosphorylated mixed lineage kinase domain-like protein; RIPK, receptor-interacting serine/threonine-protein kinase; STAT3, signal transducer and activator of transcription 3; TLR, Toll like receptor; TRIF, TIR domain-containing adaptor protein inducing IFNβ.

Taken together, various necrotic forms of RCD can be strong and potentially lethal triggers of innate immune system activation (FIG. 2a). Similar considerations apply to 'accidental' instances of necrosis that involve a large amount of cells, such as those associated with traumatic injuries[71,72]. These considerations might explain how cell death triggers solid organ rejection despite the presence or absence of immunosuppressive agents[73,74].

Inflammation triggers regulated cell death. Pro-inflammatory mediators released from necrotic cells include TNF and IFNγ, which are capable of inducing necroptosis in adjacent parenchymal cells[29,75]. The same cytokines are also released from PRR-activated infiltrating immune cells, such as macrophages, neutrophils and lymphocytes (FIG. 2b). Whereas TNF may induce death receptor-mediated necroptosis[29], IFNγ uses a signal transducer and activator of transcription 3 (STAT3)–PKR-dependent pathway to induce the interaction of RIPK1 with RIPK3 (REFS 29,75). TNF may also trigger NETosis in neutrophils and thereby fuel the vicious circle by promoting a highly immunogenic necrotic cell death subroutine. In addition to cytokine-triggered regulated necrosis, direct contact-dependent mechanisms involving immune cells may trigger necroptosis in target cells. For example, interactions via death receptors[29,76] on natural killer cells or activated $CD4^+$ T cells and $CD8^+$ T cells represent contact-dependent cell death pathways[77]. Importantly, these systems rely on an 'outside-in' signal, which may be targeted by systemically applied inhibitors or be prevented by immunosuppression. Finally, with respect to the *in vivo* setting, maximal inflammation in the form of sepsis leads to systemic hypotension, which again may cause RCD — often proceeding via MPT-RN[35,38] — that occurs as a result of hypoxia in tissues that are poorly oxygenated a priori. Altogether, these observations indicate that multiple pro-inflammatory signals can trigger RCD.

The necro-inflammatory loop. RCD, especially in its necrotic forms, and inflammation are interconnected in the context of a self-amplifying circuit that eventually results in tissue damage and organ dysfunction (FIG. 3). Such a necro-inflammatory loop originates from initial immunogenic cell death, which leads to the release of DAMPs and the induction of pro-inflammatory pathways that directly promote further necrotic RCD. Importantly, the necro-inflammatory loop is triggered in a similar fashion when cells undergo traumatic, unregulated necrosis[71,72]. The ability of RCD and unregulated necrosis to start this circuit is independent of an outside-in signal, as shown by recent data from intravital microscopy studies in the reperfusion phase after ischaemia[50]. This has led to the novel hypothesis that RCD pathways that do not depend on outside-in signalling (for example, MPT-RN and ferroptosis) may represent the initial trigger of a necro-inflammatory auto-amplification loop, in which two crucial pathophysiological features contribute to the overall organ damage: first, DAMP release will trigger inflammation (FIG. 2b); and second, in parallel, DAMPs and inflammation will trigger RCD pathways that do depend on an outside-in

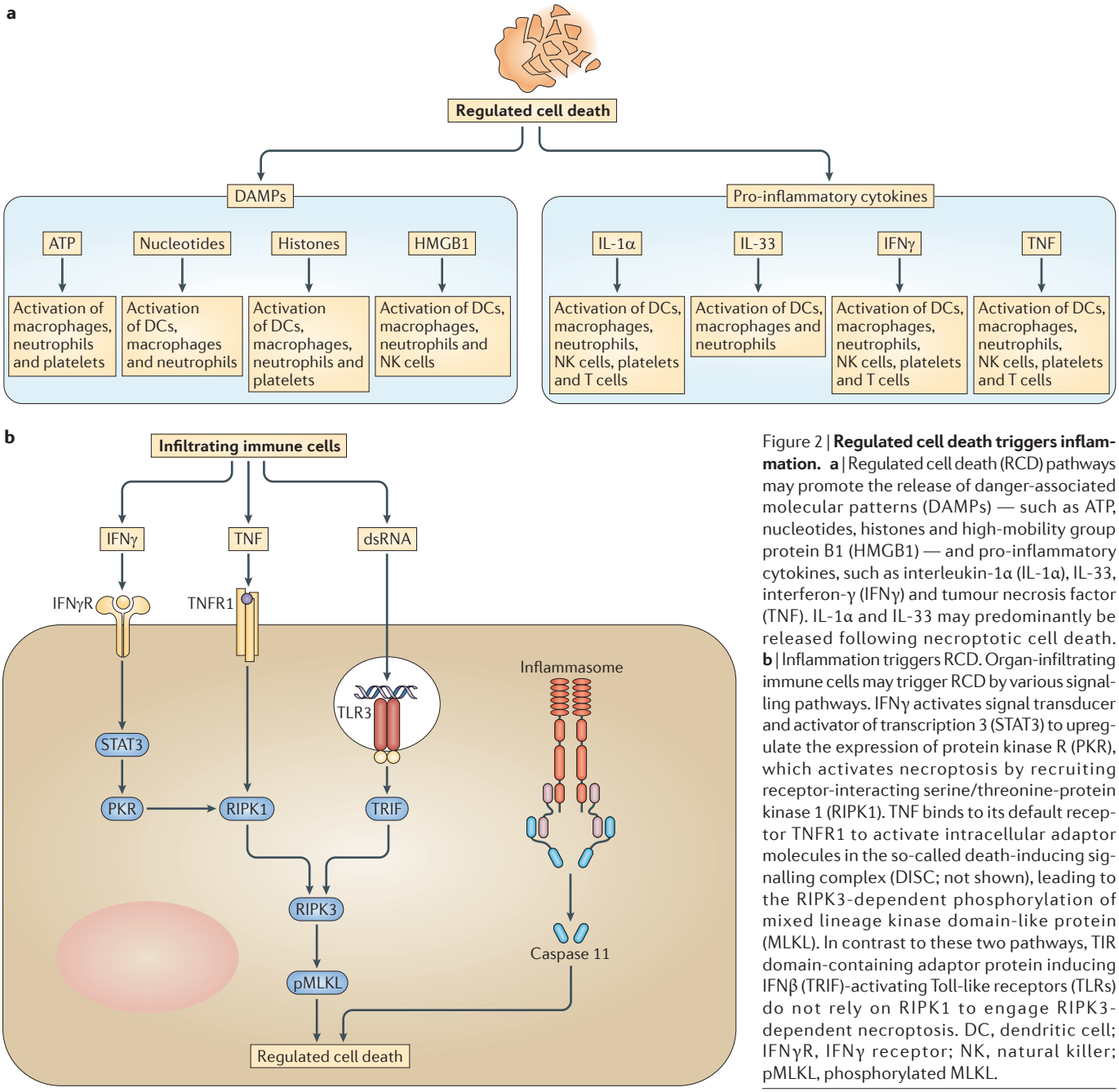

Figure 2 | **Regulated cell death triggers inflammation.** **a** | Regulated cell death (RCD) pathways may promote the release of danger-associated molecular patterns (DAMPs) — such as ATP, nucleotides, histones and high-mobility group protein B1 (HMGB1) — and pro-inflammatory cytokines, such as interleukin-1α (IL-1α), IL-33, interferon-γ (IFNγ) and tumour necrosis factor (TNF). IL-1α and IL-33 may predominantly be released following necroptotic cell death. **b** | Inflammation triggers RCD. Organ-infiltrating immune cells may trigger RCD by various signalling pathways. IFNγ activates signal transducer and activator of transcription 3 (STAT3) to upregulate the expression of protein kinase R (PKR), which activates necroptosis by recruiting receptor-interacting serine/threonine-protein kinase 1 (RIPK1). TNF binds to its default receptor TNFR1 to activate intracellular adaptor molecules in the so-called death-inducing signalling complex (DISC; not shown), leading to the RIPK3-dependent phosphorylation of mixed lineage kinase domain-like protein (MLKL). In contrast to these two pathways, TIR domain-containing adaptor protein inducing IFNβ (TRIF)-activating Toll-like receptors (TLRs) do not rely on RIPK1 to engage RIPK3-dependent necroptosis. DC, dendritic cell; IFNγR, IFNγ receptor; NK, natural killer; pMLKL, phosphorylated MLKL.

signal (that is, necroptosis, pyroptosis and parthanatos) (FIG. 3). A recently published model may allow the study of the necro-inflammatory loop in an apoptosis-deficient *Drosophila melanogaster* model, in which local wing necroptosis drives a Toll-dependent lethal systemic inflammatory response[78]. As ferroptosis and MPT-RN can be triggered by the accumulation of hydrogen peroxide, these forms of RCD probably constitute the apical trigger of the necro-inflammatory loop in the setting of ischaemia–reperfusion injury.

Implications for organ damage and cancer

Sepsis. In various models of sepsis, RIPK3-deficient mice are protected through an unknown mechanism that might involve direct necroptosis[54], or a non-necroptotic RIPK3-dependent mechanism, such as the activation of IL-1 production[10] or inflammasomes[8]. The release of DAMPs from dying cells might maintain the systemic pro-inflammatory environment but in models of sterile inflammation, if direct necrotic cell death is involved, the initial triggering event is likely to follow death receptor stimulation. Caspase inhibition by the compound zVAD shortens survival times in the TNF-mediated shock model, and RIPK3 deficiency partially protects mice in this setting[39,54,55]. It remains possible that the pathognomonic capillary leakage syndrome in sepsis might be mediated by RIPK3, but not necessarily by necroptosis (FIG. 4a). There are conflicting data regarding the role of RIPK1 in this model, as studies have used necrostatin 1 (Nec-1) and assumed that it is a specific inhibitor of the kinase domain RIPK1, which may not be the case in a strict sense[54,55,79]. Recently, the role of

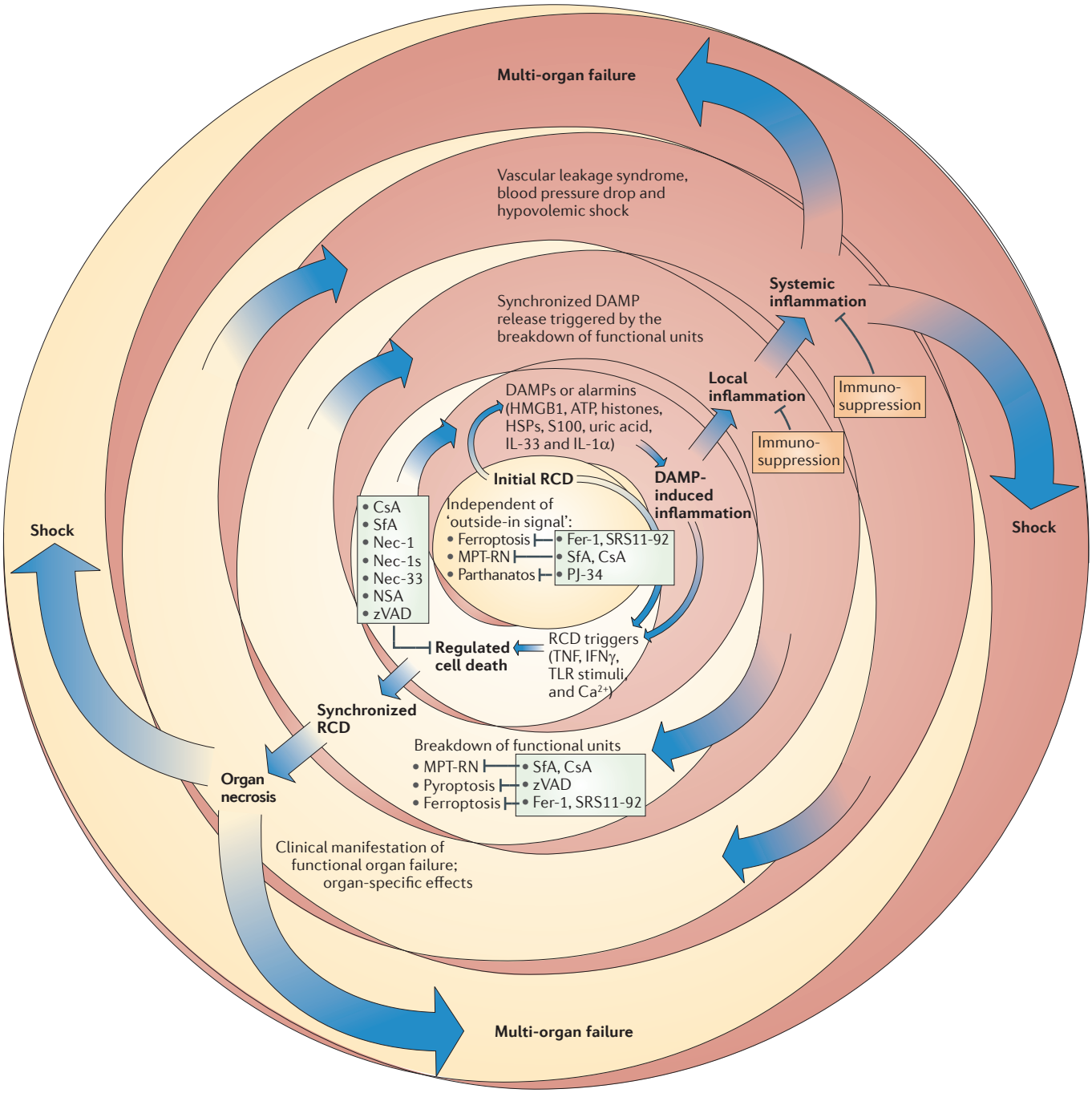

Figure 3 | **Model of the necro-inflammatory auto-amplification loop of regulated cell death and inflammation.** In hypoxic conditions, hypoperfused cells in organs or in the centre of solid tumours, regulated cell death (RCD) occurs in a manner that is independent of outside-in signalling — for example, ferroptosis, mitochondrial permeability transition-mediated regulated necrosis (MPT-RN) and parthanatos — which triggers both danger-associated molecular pattern (DAMP)-induced inflammation and outside-in signal-dependent RCD (for example, necroptosis, pyroptosis and NETosis). Given that RCD triggers inflammation and inflammation triggers regulated necrosis (FIG. 2), a necro-inflammatory auto-amplification loop may be initiated and, if it is not restrained, may lead to systemic inflammation and organ necrosis. We propose that a better understanding of the pathways that are involved in this pathophysiological response will allow specific therapeutic targeting of RCD (green boxes). However, there are currently no specific therapies targeting cell death that are anywhere close to being used the clinic, with the exception of cyclosporine A (CsA), which is both a strong immunosuppressant and an inhibitor of MPT-RN. In general, interference with the auto-amplification loop should ideally target this spiral as close to the epicentre of the inflammatory response as possible to achieve maximal organ protection. Fer-1, ferrostatin 1; HMGB1, high-mobility group protein B1; HSP, heat shock protein; IFN, interferon; IL, interleukin; MPT, mitochondrial permeability transition; Nec, necrostatin; NSA, necrosulfonamide; PJ-34, poly(ADP-ribose) polymerase 1 inhibitor; SfA, sanglifehrin A; SRS11-92, inhibitor of ferroptosis; TLR, Toll-like receptor; TNF, tumour necrosis factor; zVAD, pan-caspase inhibitor.

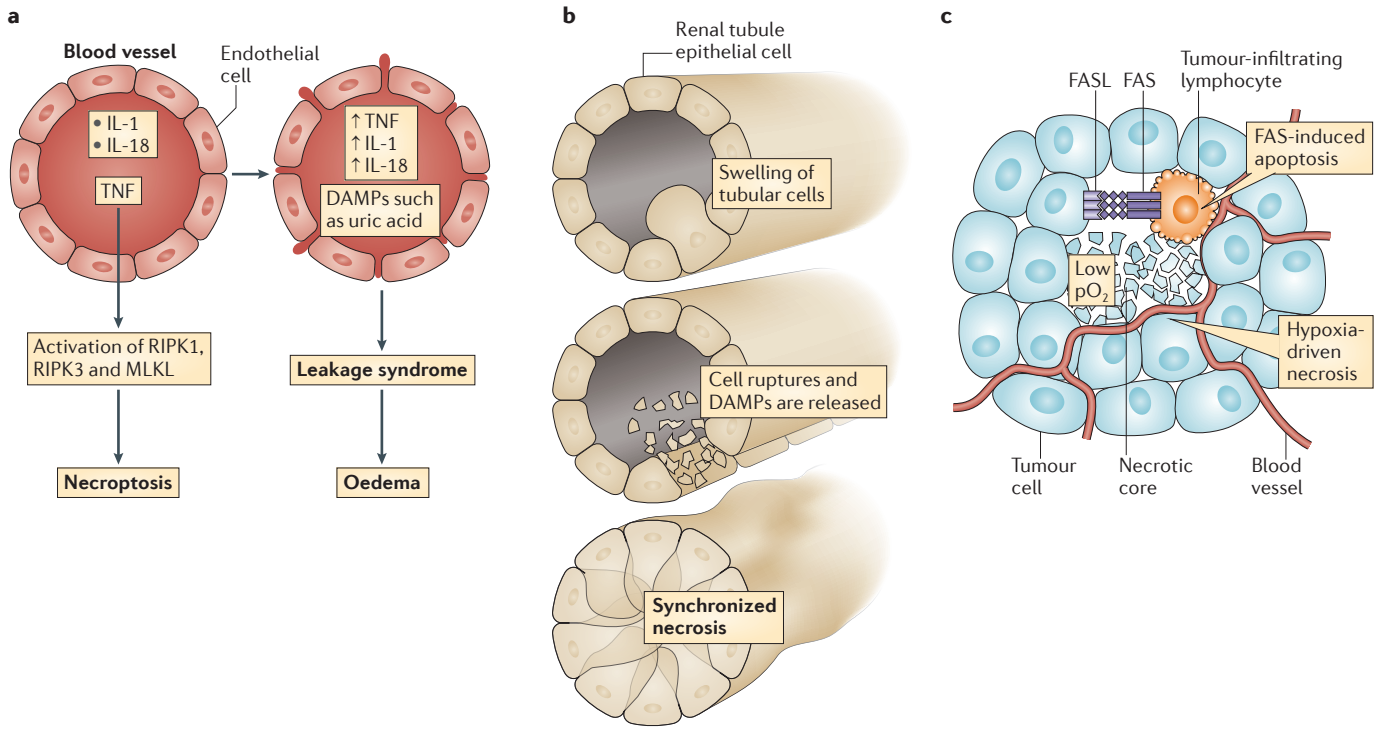

Figure 4 | **The auto-amplification loop of regulated cell death and inflammation in sepsis, ischaemia–reperfusion injury and solid cancer.** The auto-amplification loop may drive vascular leakage syndrome, oedema formation, systemic inflammatory response syndrome (SIRS) and sepsis, ischaemia–reperfusion injury and cancer. **a** | In SIRS that is initiated by intravenous administration of recombinant tumour necrosis factor (TNF), pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18, together with TNF, induce necroptosis of endothelial cells and thereby aggravate the vascular leakage syndrome in manner that is dependent on receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL). During necroptosis, uric acid is one of the most important danger-associated molecular patterns (DAMPs) that further fuels the auto-amplification loop and it has been shown to be associated with a poor outcome. **b** | In ischaemia–reperfusion injury, epithelial cells swell in response to the induction of regulated necrosis pathways — such as ferroptosis, necroptosis and others — until these cells rupture and release DAMPs that initiate the inflammatory response. In the example of renal tubules, which represent multicellular functional units, synchronized cell death is subsequently triggered by as yet undefined mechanisms, but an important pathophysiological role has been ascribed to ferroptosis. **c** | In cancer, central hypoxia-driven regulated necrosis may generate a steep oxygen pressure gradient that allows the tumour to grow rapidly following the proliferation of cells on the tumour surface, which is the interface with the immune system. Mitochondrial permeability transition-mediated regulated necrosis (MPT-RN) and necroptosis may mediate this central necrosis and thereby provide the oxygen gradient, in line with the finding that some adenocarcinomas have activating mutations in *MLKL* which drive necroptosis in these cells[104]. In contrast to the cells at the tumour surface, and in contrast with the hallmarks of cancer (prevention of regulated cell death), cells in the centre of tumours might actively undergo necrosis, suggesting that the prevention of this process might have therapeutic potential. However, unlike the central necrosis, tumour-infiltrating lymphocytes are cleared by engagement of the FAS–FAS ligand (FASL) system, which in some cancers leads to survival and proliferation of the tumour cells and apoptosis of lymphocytes. IL, interleukin; pO_2, partial oxygen pressure.

caspase 1 and caspase 11 have been distinctly evaluated in an elegant study that used several sepsis models, demonstrating the importance of IL-1β and IL-18 in the progression of the disease, and highlighting therapeutic opportunities that are downstream of inflammasomes[80]. Once sepsis is evident, dying cells have been shown to release uric acid from intracellular stores but, more importantly, the vast majority of uric acid originates from post-mortem nucleic acid degradation, probably promoting even more cell death[81].

Acute crystallopathies. Crystals are defined as solid structures with molecules, atoms and ions arranged in patterns, and they are recognized as the causative trigger for diverse diseases[82]. In gout, the rapid onset of intense inflammation is triggered by uric acid crystals that activate the NOD-, LRR- and pyrin domain-containing 3 (NLRP3; also known as NALP3) inflammasome[83], and similar mechanisms have been described for calcium oxalate crystals[84], atherogenesis by cholesterol crystals[85], and hydroxyapatite-associated arthropathy[86]. The NLRP3 inflammasome was associated with the innate immune response to amyloid-β, silica crystals and aluminium salts[87,88]. In most of these models, RCD is of central relevance as crystals (in particular, monosodium urate crystals) induce phagocyte death, which triggers neutrophil recruitment and NETosis[89]. NETosis itself may involve the release of pro-inflammatory monosodium urate crystals that trigger inflammation[90], setting up the auto-amplification loop that occurs in patients with acute gout. Disrupting this loop of necrosis and inflammation by inhibiting crystal uptake and neutrophil recruitment — using the toxic alkaloid colchicine[91] or by interference with IL-1β signalling[92] — is very effective at preventing the disease process. However, it was reported that NETosis can unexpectedly suppress inflammation in certain circumstances[93]. Taken together, these data strongly suggest that crystals are capable of directly inducing necrotic subroutines of RCD, but strong evidence for this hypothesis is missing.

Ischaemic injury. In recent years, research on ischaemia–reperfusion injury has greatly contributed to our understanding of RCD pathways[39,50,94], and as with cancer (see below), there is an unmet clinical need for novel therapies for this type of injury. Necroptosis[50], MPT-RN[94] and parthanatos[94] have been ascribed important pathophysiological functions in ischaemia–reperfusion injury, and the relative contribution of the known pathways of regulated necrosis to this type of injury has been extensively discussed, but requires further investigation[14,50] (FIG. 4b). Studies in isolated renal tubules — which serve as organoids for the *ex vivo* analysis of multicellular functional organ-like units — however, suggest that glutathione crucially regulates iron-dependent cell death[95], a typical feature of ferroptosis which has recently been detected in this model[96]. Unlike necroptosis, which relies on an outside-in signal[2], ferroptosis may be triggered in the reperfusion phase after ischaemic periods and lead to the accumulation of peroxides owing to the lack of sufficient glutathione for maximal activity of GPX4 (REF. 45). The independence of an outside-in signal renders ferroptosis one candidate pathway that may cause the initial necrosis which triggers the auto-amplification loop (FIG. 3). However, the initial regulated necrosis pathway may be independent of an outside-in signal, but the subsequent amplification apparently does include necroptosis.

The necro-inflammatory loop in cancer. Recently, there have been major breakthroughs in trials of immunotherapy for cancer[97]. Several conventional anticancer agents have been shown to exert immunostimulatory effects, which may explain the clinical efficacy of these drugs[98]. Activating regulated inflammatory cell death pathways could stimulate endogenous antitumour immune responses, but the potential role of tumour-infiltrating lymphocytes in promoting tumour growth also needs to be considered[99]. Some existing chemotherapeutic agents activate inflammatory cell death (for example, temozolomide in glioblastoma), perhaps explaining the unique efficacy of such agents compared to other apoptosis-inducing chemotherapies[98].

When RAS-transformed tumour cells were used to screen for selectively lethal compounds, erastin was among the strongest hits[42]. This ferroptosis inducer might be suited to induce a local necro-inflammatory environment in highly metabolically active tissues, such as cancers[43,44]. As with the events of ischaemia[96], renal tubular clear cell carcinomas seem to be particularly susceptible to ferroptosis[45], and this type of cancer has also been suggested as a candidate for immunotherapy[100].

Importantly, activating mutations in *MLKL* have been described[101], some of which cause conformational changes in the N-terminal bundle–brace motif[26] and thereby eventually induce necroptosis in tumour cells[28]. However, one of the reported mutations in *MLKL* does not affect the necroptotic response, raising the possibility that MLKL may have additional neoplastic effects[26] that are mediated by other pathways. However, it is tempting to speculate on a pro-necrotic role of MLKL in generating the necrotic core of some solid tumours.

PARP1 has been recognized as a target for cancer therapy for decades. It is noteworthy that PARP1 inhibitors not only stabilize DNA but also prevent RCD in the form of parthanatos[102]. As parthanatos has been demonstrated to be of importance in organ failure in several models of ischaemia, an intriguing hypothesis emerges: the central necrosis may drive an oxygen gradient and thereby provoke neovascularization that provides the cells at the surface of the tumour with nutrients that are needed for rapid tumour expansion (FIG. 4c). It is tempting to speculate that inhibition of regulated necrosis might be beneficial in such settings. Although the resistance of tumour cells to cell death — particularly those cells at the surface of a tumour — is one of the hallmarks of cancer[103], this feature is lost in the cells that form the necrotic core of a tumour.

Cell death as a therapeutic target

Are immunosuppression and interference with RCD two sides of the same coin? Pharmacological inhibition of the auto-amplification loop of regulated necrosis and inflammation should ideally target the initial necrosis, perhaps ferroptosis, and thereby theoretically prevent the loop from developing. Targeting ferroptosis *in vivo* has been difficult owing to the instability of ferrostatin 1 in the serum. In organotypic cultures (in which cells are grown in a three-dimensional environment) and primary renal tubules, ferrostatins have shown tissue-protective effects to an extent that has not yet been achieved by any other compound[96]. Second-generation and third-generation ferrostatins are currently being developed.

Clinically, patients will present with ischaemic, toxic or septic injury when the auto-amplification loop has progressed beyond the initial necrosis. Therefore, prevention of regulated necrosis and immunosuppression are the general means to be applied in such situations. Many agents could be used to prevent the necrotic component of the loop including: inhibitors of necroptosis (such as Nec-1 (REFS 39,62), Nec-1s, Nec-33, necrosulfonamide[23], and probably some drugs that are already in clinical use); MPT-RN (such as sanglifehrin A and cyclosporine A[39]); ferroptosis (such as ferrostatin 1 (REF. 43) and SRS11-92 (REF. 96)); and also caspase inhibitors and inflammasome inhibitors (such as pralnacasan (VX-740; Vertex Pharmaceuticals) and belnacasan (VX-765; Vertex Pharmaceuticals)) that target pyroptosis[18] (FIG. 3). A combination therapy approach could involve the use of such agents alongside standard immunosuppression (for example, the use of steroids, mycophenolate and rapamycin) to further interfere with regulated necrosis. Importantly, the widely used calcineurin-inhibitor cyclosporine A additionally inhibits MPT-RN[29,30,39]. This may explain the great success of this drug in solid organ transplantation over decades. Cyclosporine A might therefore be the only known compound that blocks cell death and is also immunosuppressive, thereby inhibiting both sides of the necro-inflammatory loop.

Summary and perspectives

It is important that we realize the possibility of interfering with regulated necrosis in patients. Our growing understanding of the molecular pathways of regulated necrosis will allow the research community to develop small molecules that block this amplification loop. Combination therapy that targets both regulated necrosis and inflammation is therefore likely to yield strong beneficial effects in a wide range of common clinical disorders, such as stroke, myocardial infarction, cancer and pancreatitis. Furthermore, such an approach may help to solve other clinical problems, including solid organ transplantation and pharmacological toxicities. Importantly, combination therapy is well established in solid organ transplantation, but exclusively focuses on immunosuppression. Despite the many inhibitors that are available, RCD pathways, have not been yet been targeted clinically.

The interdisciplinary community of clinicians, immunologists and cell death researchers faces several open questions that ought to be urgently addressed. What may be the drawbacks of blocking regulated necrosis? How long do we need to treat patients for? Will we induce impaired host defence or even cancer by preventing

regulated necrosis? Will the most promising scenario with the best benefit-to-risk ratio to carry out the first clinical trials be solid organ transplantation? Although we know that we can interfere with inflammation by using powerful and specific immunosuppressive agents, such questions should motivate scientists at the edge of translational research to invest any possible effort into addressing an area that has been neglected for at least a century. We argue that it is now time to prevent necrosis.

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Competing interests statement

The authors declare no competing interests.

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