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Sensing of cell death by myeloid C-type lectin receptors

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Abstract

Molecules associated with dead or dying cells can be detected by receptors on macrophages and dendritic cells. Signals from these receptors impact myeloid cell function and play a role in determining whether death is silent or proinflammatory, tolerogenic or immunogenic. Prominent among myeloid receptors detecting dead cells are C-type lectin receptors (CLRs). Signals from these receptors variably induce endocytosis of cell corpses, corpse degradation, retrieval of dead cell-associated antigens and/or modulation of immune responses. The sensing of tissue damage by myeloid CLRs complements detection of pathogens in immunity and represents an ancient response aimed at restoring tissue homeostasis.

Introduction

Cell death occurs continuously in our bodies as a consequence of tissue remodelling, injury or infection. There are a number of cell death modalities, traditionally distinguished by morphological criteria but, more recently, classified on the basis of death-inducing molecular pathways [1]*. Independently of modality, cell death generates corpses that need to be removed in order to maintain tissue integrity. Myeloid cells, in particular those of the mononuclear phagocyte family, are important scavengers of dead and dying cells. But they do more than act as undertakers. Myeloid cells possess receptors that detect molecules released from dying cells or exposed by cell corpses and can integrate signals from those receptors to either suppress or induce inflammation. In addition, signals from dying or dead cells impact a particular type of mononuclear phagocyte, the dendritic cell (DC), allowing it to retrieve antigens from dead cell corpses and present them for T cell perusal in either an immunogenic or tolerogenic context. Different forms of cell death can often be mapped onto distinct immune outcomes (Box 1). Another way to interpret innate recognition of cell death is to focus away from the death process onto the receptors that are utilised by myeloid cells to recognise dead or dying cells. Prominent among such receptors are members of the C-type lectin receptor (CLR) superfamily (Table 1). Myeloid CLRs involved in dying or dead cell detection include for example Lox-1 (OLR1) [2] and Mgl-1 (Clec10a) [3], which detect

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ligands in apoptotic cells, or Mincle (CLEC4E) [4]** and DNNGR-1 (CLEC9A) [5]**, which sense “damage-associated molecular patterns” (DAMPs) exposed or released by necrotic cells (Table 1; see also Box 1). These CLRs are all endocytic receptors expressed by macrophages and DCs and implicated in corpse scavenging, degradation or antigen salvage pathways. They exert their functions by mediating corpse uptake, regulating endocytic traffic or signalling to modulate gene expression. CLRs can thus play a major role in determining whether death sensing by myeloid cells is immunologically silent or results in an innate and/or adaptive immune response [6]*.

Myeloid C-type lectin receptors sensing damaged self

The C-type lectin-like domain (CTLD) [7] is a conserved structural motif that has evolved to adapt to a variety of ligands. Most commonly, CTLDs are involved in calcium-dependent carbohydrate binding, but many also bind glycans, proteins or lipids in a calcium-independent manner [8]. Myeloid cells express a variety of integral membrane CLRs that signal to induce or modulate endocytosis, microbicidal activity or gene transcription [9]. Many CLRs can sense “pathogen associated molecular patterns” (PAMPs; non-self) but they can also be involved in cell adhesion and communication or can bind neoglycans expressed by transformed cells (altered self). A small group of myeloid CLRs can detect a variety of ligands that are exposed or released from dying or dead cells (Table 1). As such, these CLRs can be seen as innate sensors of damaged self. For example, DEC-205 (Ly75) can act as a scavenger receptor for oxLDL [10], and DEC-205-IgG fusion proteins have been shown to bind to both apoptotic and necrotic cells although the exact nature of the ligand is not known [11]. The recent finding that DEC-205 binds phosphorothioate-linked DNA oligonucleotides [12] suggests that one ligand might be cell-derived nucleic acids. Mgl1 binds to galactose-containing Le^X and Le^A glycans [13], which might be exposed in apoptotic cells and explain the ability of the latter to be recognised by recombinant Mgl1 [3]. Similarly, LOX-1 binds to aged and apoptotic cells [2] perhaps because such cells expose oxidised lipids, Hsp-60 or Hsp-70, all of which have been identified as LOX-1 ligands [14,15]. Mincle and DNNGR-1 do not bind to apoptotic cells but to primary and secondary necrotic cells that have lost membrane integrity [4]**[5]**. This is because both receptors recognise ligands that are exclusively present within the cell rather than exposed at the plasma membrane. The ribonucleoprotein SAP-130 is the necrotic cell-derived ligand for Mincle [4]**, whereas F-actin filaments exposed by necrotic corpses act as the ligand for DNNGR-1 [16]*[17]*. Interestingly, most of the CLRs involved in dead cell recognition also have non-self ligands (Table 1). For example, in addition to SAP-130, Mincle binds to α -mannose in fungal species of *Malassezia* and some *Candida* strains, and to the mycobacterial glycolipid trehalose-6,6'-dimycolate (TDM) [18-20]¹. The exception is DNNGR-1, for which no PAMP ligand has been identified to-date (although, of course, F-actin is also found in fungi and parasites).

Most CLRs possess tyrosine-based, triacidic or dileucine intracellular motifs that mediate endocytosis and direct the receptors to distinct endosomal compartments [9]. In DEC-205

¹The microbial ligands for Mincle bind to the carbohydrate recognition domain of the receptor in a calcium-dependent manner, in contrast to SAP-130, whose binding involves a distinct site and does not require calcium [4]**[20, 21].

and Mgl-1, these motifs target the receptors and their cargo to late endosomes/lysosomes, whereas in LOX-1 they direct the receptor into an early endosomal compartment (Table 1, Figure 1). All of these CLRs have in common the fact that they act primarily as uptake receptors and help mediate clearance and degradation of corpses by myeloid cells. In contrast, the CLRs recognising necrotic cells appear to have functions other than corpse phagocytosis (see below) and thus should not be considered principally as uptake receptors. Consistent with this notion, DNNGR-1 does not mediate particle uptake when expressed in a non-phagocytic cell line [22] and is redundant for uptake of dead cells by DCs [5]. Similarly, Mincle localises to phagocytic cups during the interaction with its ligand in *Candida albicans*, but it is not essential for fungal uptake [19].

Decoding the antigenicity of dead cells

In vertebrates, proteins within corpses can be a valuable source of antigens for priming T cells, especially if the dying cells are cancerous or infected with a pathogen. It is therefore important that DCs possess mechanisms to preserve antigenic information present in cell corpses. Consistent with this notion, some CLRs expressed by DCs appear to function primarily by regulating dead cell antigen retrieval and presentation (Fig. 1). For example, DNNGR-1 diverts endocytic cargo to a poorly degradative recycling endosomal compartment characterised by expression of EEA1, Rab5a and Rab11 [23]** (Figure 1). This compartment appears similar to that targeted by the mannose receptor, another CLR [24], and has limited acidification potential, preventing proteolytic activity and favouring only partial degradation of antigens. This makes the latter suitable substrates for MHC class I cross-presentation [24-26]. Notably, the absence of DNNGR-1 decreases cross-presentation of dead-cell associated antigens by the CD8 α^+ family DCs, which express the receptor [5,23]**[27]**. This decrease in cross-presentation can be reversed by blockade of lysosomal acidification or lysosomal proteases, confirming that DNNGR-1 acts primarily by protecting antigens from lysosomal degradation [27]**.

DNNGR-1 modulation of antigen processing could potentially affect equally cross-tolerance or cross-priming. However, DNNGR-1 deficiency does not affect cross-tolerance in a transgenic mouse model [23]**. This might indicate the fact that the substrates for cross-tolerance are primarily contained within apoptotic cells, which do not expose ligands for DNNGR-1. The prediction is that DNNGR-1 would therefore be involved only when primary or secondary necrosis are at play, normally in the context of a pathological setting. One of these settings is cytopathic infection. Recent results show that DNNGR-1-deficient mice display an impairment in CTL priming to viral antigens in models of cytopathic infection with vaccinia or herpes simplex virus [27]**[23]**. Notably, in these infections, there is abundant generation of viral PAMPs, which indicates that DNNGR-1 regulation of cross-presentation is not superseded by DC stimulation via PAMP receptors [27]**[23]**. These observations suggest a novel and non-redundant point in control of immunity to infection, in which DAMP receptors such as DNNGR-1 mark dead cells as substrates for antigen processing and presentation, whereas PAMP receptors (e.g., TLRs) detect signs of infection in the damaged cells and promote DC activation². This is relevant for vaccination as it suggests that tissue damage signals could be used to enhance antigen cross-presentation, helping elicit CTL responses.

Other CLRs may participate in decoding the antigenicity of dying or dead cells although in many cases it is unclear if this reflects a function of the CLR in regulating antigen processing or simply its ability to promote dead cell uptake and, therefore, mediate antigen capture. For example, coupling of Hsp-70 or Hsp-60 to a model antigen favours its binding to DCs and cross-presentation to CD8⁺ T cells, and this process is inhibited using blocking anti-LOX-1 antibody [14,15]. LOX-1 could therefore potentially mediate cross-presentation of apoptotic cell-associated antigens. Supporting this hypothesis, blockade of LOX-1 on IFN- α -conditioned human monocyte-derived DCs reduces apoptotic cell uptake by DCs and CD8⁺ T cell cross-priming against apoptotic cell-associated antigens [30]. DEC-205 also mediates uptake of antigens and directs them preferentially to a late endosomal/lysosomal compartment that favours MHC class II loading [31]. Although the impact of DEC-205 on presentation of dead cell-associated antigens remains unclear, its capacity and that of other myeloid CLRs (e.g., DNGR-1) to promote (cross-) presentation of antigen-bearing cargo makes them attractive targets in vaccination [32-34].

Immune modulation by C-type lectin receptors sensing damaged cells

Independently of uptake and endocytic traffic regulation, CLRs can act as signalling receptors to induce or modulate gene expression in myeloid cells in response to dead cell encounter (Figure 2) [9]. There is not much information on the signalling pathways and functional outcomes downstream of CLRs sensing of apoptotic cells. In a model of colitis, Mgl1 interaction with its ligands induces IL-10 production by lamina propria macrophages [35]. Mgl1-deficient mice develop more severe inflammation than controls [35] and it is therefore possible that some of the anti-inflammatory effects of apoptotic bodies might be mediated through Mgl1. More is known about signalling by receptors for necrotic cells and its functional consequences. DNGR-1 couples to Syk through an intracellular hemITAM, similar to that of Dectin-1, a fungal PAMP receptor³, and phospho-Syk is found at the contact area between DCs and dead cells in a DNGR-1-dependent fashion [5]. Syk signalling downstream of Dectin-1 results in activation of CARD9-NF- κ B, MAPKs and NFAT, leading to transcriptional activation in DC and macrophages [9]. However, there is no evidence that DNGR-1 can trigger these pathways in DCs. DNGR-1 does not activate DCs upon interaction with dead cells or following engagement of a chimeric receptor with a Dectin-1 ectodomain fused to DNGR-1 [23]**. Moreover, DNGR-1 does not modulate DC activation induced by viral PAMPs present within virus infected dead cells [23]**[27]**. Thus, DNGR-1 sensing of cell death appears to affect mainly the processing and fate of the cargo without inducing expression of genes encoding pro-inflammatory mediators (Fig. 2).

In contrast, Mincle associates with the ITAM-bearing FcR γ chain through an arginine residue in the transmembrane region [4]**. TDM stimulation of macrophages reveals that Mincle activates the FcR γ /Syk/CARD9/ NF- κ B axis, leading to proinflammatory cytokine (TNF- α , IL-6), chemokine (CXCL1, CXCL2) and nitric oxide production [21,37]. Similarly, SAP-130 induces MIP-2 production via Mincle and exposure to dead cells triggers MIP-2

²TLRs also regulate antigenicity in some instances. For example, recognition of viral dsRNA within dying infected cells by TLR3 [28] or recognition of HMGB1 on dying tumour cells by TLR4 contribute to efficient processing and cross-presentation of dead cell-associated antigen [29].

³It has been argued that Dectin-1 also binds to apoptotic cells [36], although other studies have failed to confirm this [5]**.

and TNF- α production in macrophages, which can be inhibited by a blocking anti-Mincle antibody [4]**. One consequence of pro-inflammatory cytokine and chemokine production following Mincle engagement by SAP-130 is the attraction of neutrophils. Indeed, dead cell-induced neutrophilia is severely impaired in mice treated with an anti-Mincle blocking antibody [4]**. Thus, Mincle sensing of dead cells by macrophages promotes neutrophilia, which contributes to corpse clearance and tissue repair. Because of this effect, it is possible that Mincle also contributes to pathological or chronic inflammation in some instances. It is interesting to note that Mincle is upregulated in patients with rheumatoid arthritis [38].

Concluding remarks

Some myeloid CLRs recognise oxidised lipids, heat shock proteins, F-actin filaments or ribonucleoproteins, all of which can be exposed by apoptotic and/or necrotic cells. Detection of cell damage by these CLRs regulates myeloid cell function and affects clearance of cell corpses, presentation of corpse-associated antigens or induction of inflammation and tissue repair. CLRs sensing apoptotic cells (Mgl1, DEC205 and LOX-1) are active at mediating corpse uptake for disposal or for antigen retrieval, but do not have a defined role in immune modulation, even though signals from Mgl1 may dampen inflammation. In contrast, CLRs sensing necrotic cells do not primarily promote corpse clearance but detect DAMPs. They signal to initiate inflammatory processes leading to tissue repair (Mincle) or to promote cross-presentation of dead cell-associated antigens (DNGR-1). The characterisation of CLRs that sense cell death and regulate antigenicity and inflammation reveals how the immune system integrates damage and infection signals and offers a new axis for intervention in autoimmune diseases or vaccination.

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Box 1**Immune consequences of sensing cell death**

Immunologists have attempted to map cell death modalities onto the effector responses of mononuclear phagocytes and immunological outcome. For example, apoptosis is generally seen as a silent or anti-inflammatory process that additionally results in induction of T cell tolerance to apoptotic cell-associated antigens [39]. However, certain drugs can induce a form of tumour cell apoptosis that is both pro-inflammatory and immunogenic and is associated with translocation of calreticulin from the endoplasmic reticulum to the plasma membrane and release of oxidized HMGB-1 and ATP [6]*. In addition, apoptotic cells that are not rapidly cleared by their neighbours or phagocytes undergo a disintegration process termed secondary necrosis. Both secondary and primary necrosis, effectively defined as an irreversible loss of plasma membrane integrity, are typically considered inflammatory and immunogenic because they allow release of pro-inflammatory cell constituents that are normally sheltered from innate surveillance by virtue of their intracellular localisation. Such “damage-associated molecular patterns” (DAMPs) released or exposed by necrotic cells, include uric acid, HMGB1, ATP, SAP-130 and F-actin, [4,16,17,40-43]. However, DAMPs are not always pro-inflammatory and, indeed, necrotic cell death has also been reported to be immunologically silent, anti-inflammatory or tolerogenic [44-47]. The immunological consequences of dead cell encounter are often studied from the perspective of antigen-specific T cell immunity but it is important to note that DAMP-induced sterile inflammation is an ancient process conserved in invertebrates, which lack an adaptive immune system [48]. As such, DAMP release by necrotic cells acts as a marker of tissue injury and its pro-inflammatory properties are likely to be linked to the process of tissue repair, for example promoting an influx of neutrophils to clean up wounds [49]. When dysregulated, sterile inflammation can become chronic and contribute to human diseases as diverse as atherosclerosis, cancer and neurodegeneration.

Despite its origins as a tissue repair process, it is clear that necrosis, in vertebrates, can impact adaptive immunity when it is coupled to the presence of neo-antigens such as following infection or tumourigenesis [6]*. Necrosis may additionally contribute to autoimmunity [50]. Notably, recent findings have revealed that necrosis can be a form of programmed cell death rather than the accidental “explosion” of cells following injury or lack of apoptotic corpse clearance. Such programmed necrosis (necroptosis) can be seen in response to infection and is likely to be pro-inflammatory and immunogenic [51]. Finally, pro-inflammatory cell death can additionally take the form of pyroptosis, a type of cell demise resembling necrosis and accompanied by release of IL-1 β often seen in macrophages infected with intracellular bacteria. Notably, during infection one needs to consider the dual effects of dead cell and pathogen recognition by myeloid cells on immunological outcome. For example, infected apoptotic cells sensed by myeloid cells trigger the production of TGF- β together with IL-6 due to sensing of dead cells and “pathogen-associated molecular patterns” (PAMPs), respectively. This unusual

combination of anti-inflammatory and pro-inflammatory cytokines favours the generation of a Th17 response [52].

Highlights

- Some C-type lectin receptors (CLRs) are involved in uptake of apoptotic cells.
- Other CLRs detect necrotic cells and signal to initiate or modulate inflammation and immunity.
- CLRs can direct dead cell cargo to distinct endocytic compartments.
- DNGR-1 decodes the antigenicity of necrotic cells by regulating crosspresentation of dead cell-associated antigens.
- Mincle sensing of necrotic cells promotes inflammation and neutrophilia.

Figure 1. CLR control of uptake and degradation of cell corpses

CLRs regulation of uptake of apoptotic (Mgl1, Lox-1 or DEC-205) or necrotic cells (DEC-205 or DNGR-1). DNGR-1 and LOX-1 promote localisation of cargo to an early endosome compartment. In particular, DNGR-1 sequesters cargo in a poorly degradative early endocytic compartment that favours class I cross-presentation. Mgl1 and DEC-205 primarily deliver cargo to a late endosome-lysosomal compartment, which is best suited for MHC class II presentation.

Figure 2. Immune modulation by CLRs sensing damaged cells

Both DNGR-1 and Mincle signal via Syk kinase in response to encounter with necrotic cells. Mincle/Syk signalling triggers the CARD9 axis that results in NF- κ B activation and production of proinflammatory cytokines and chemokines. In contrast, Syk signalling downstream of DNGR-1 does not couple to NF- κ B in DC for reasons that remain unclear but have to do in part with the aminoacid composition N-terminal to the tyrosine in the

hemITAM motif [23]**. Detection of apoptotic cells by Mgl1 can result in an anti-inflammatory response via an unknown signalling pathway.

Table 1

Myeloid C-type lectin receptors sensing damaged self

Myeloid CLRs reported to interact with damaged cells discussed in this review. Abbreviations: aa: alternatively activated; B: B cell; DC: dendritic cell; EC: endothelial cell; GalNAc: N-acetyl-galactosamine; Hs: Homo sapiens; LC: Langerhans cell; Le: Lewis; mDC: myeloid DC; MDCC: monocyte-derived DC; MØ: macrophage; Mm: Mus musculus; ND: none detected; ODN: oligodeoxynucleotide; PDC: plasmacytoid DC; PLA: plasminogen activator; PMN: polymorphonuclear leukocyte; tDC: tissue DC; tEC: thymic epithelial cells.

Common name(s)	Gene name	Expression	Endocytic activity	Functional effects	Ligand/s	Ligand origin		References
						Exogenous	Endogenous	
DEC205, CD205	LY75 (Hs) Ly75 (Mm)	DC, LC, tEC, B, MØ	Late endosome-lysosome	corps uptake; antigen presentation	PLA (<i>Y. pestis</i>) K12 (<i>E. coli</i>) Class B CpG ODN	HIV-1 <i>Y. pestis</i> <i>E. coli</i>	apoptotic cells, oxLDL	[10-12, 31, 32]
Mgl1, Mgl, CD301a (Mm)	Clec10a (Mm)	DC, aaMØ	Late endosome-lysosome	corps uptake ; ↑ IL-10	Le ^x , Le ^A Terminal galactose, GalNAc,	<i>Streptococcus</i> spp. <i>Lactobacillus</i> spp	apoptotic cells, sialoadhesin	[3, 13, 35]
LOX-1	OLR1 (Hs) Olr1 (Mm)	EC, mDC, MDCC, B, MØ	early endosomes	corps uptake; antigen capture and presentation.	Hsp-60, Hsp-70, oxidized lipids	<i>E. coli</i> <i>S. aureus</i>	apoptotic/ aged cells, oxLDL, oxidized lipids	[2, 14, 15]
DNGR-1	CLEC9A (Hs) Clec9a (Mm)	Mm: CD8α ⁺ DC, CD103 ⁺ CD11b ⁻ tDC, PDC (low) Hs; BDCA3 ⁺ DC	Early & recycling endosomes	Necrotic cell antigen cross- presentation	F-actin	ND	necrotic cells	[5, 16, 17, 22, 23, 27, 33, 34]
Mincle	CLEC4E (Hs) Clec4e (Mm)	MØ, PMN	ND	↑ TNF-α, IL-6, CXCL1, CXCL2	α-mannose, glycolipids, SAP-130.	<i>M. tuberculosis</i> <i>C. albicans</i> <i>Malassezia</i> spp.	necrotic cells	[4, 18-21]