Seminars in Immunology 66 (2023) 101726

Contents lists available at ScienceDirect

Seminars in Immunology

journal homepage: www.elsevier.com/locate/ysmim

DNGR-1-mediated cross-presentation of dead cell-associated antigens

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ARTICLE INFO

Keywords:
DNGR-1/CLEC9A
Dendritic cells
Cross-presentation
Cross-priming
CD8$^+$ T cells
Anti-viral immunity
Anti-tumour immunity
Adaptive immunity

ABSTRACT

Conventional dendritic cells type 1 (cDC1) are critical for inducing protective CD8$^+$ T cell responses to tumour and viral antigens. In many instances, cDC1 access those antigens in the form of material internalised from dying tumour or virally-infected cells. How cDC1 extract dead cell-associated antigens and cross-present them in the form of peptides bound to MHC class I molecules to CD8$^+$ T cells remains unclear. Here we review the biology of dendritic cell natural killer group receptor-1 (DNGR-1; also known as CLEC9A), a C-type lectin receptor highly expressed on cDC1 that plays a key role in this process. We highlight recent advances that support a function for DNGR-1 signalling in promoting inducible rupture of phagocytic or endocytic compartments containing dead cell debris, thereby making dead cell-associated antigens accessible to the endogenous MHC class I processing and presentation machinery of cDC1. We further review how DNGR-1 detects dead cells, as well as the functions of the receptor in anti-viral and anti-tumour immunity. Finally, we highlight how the study of DNGR-1 has opened new perspectives into cross-presentation, some of which may have applications in immunotherapy of cancer and vaccination against viral diseases.

1. Introduction

Antigen presentation by conventional dendritic cells (cDCs) is fundamental for initiating and propagating adaptive immunity[1,2]. The antigen processing machinery leading to peptide presentation by major histocompatibility complex class II proteins (MHC-II) is found almost exclusively in endosomes or phagosomes, suggesting that MHC-II is biased towards presentation of exogenous antigens, i.e., extracellular derived proteins acquired by cDCs via endocytosis or phagocytosis [3,4]. In contrast, MHC class I (MHC-I) peptide loading occurs in the endoplasmic reticulum (ER) and is often biased towards presentation of endogenous antigens, i.e., those synthesised within the cell [3,5]. Canonical MHC-I antigen processing involves the proteasome, which degrades proteins in the cytosol into small peptides that translocate into the ER through a transmembrane complex called transporter-associated with antigen-processing (TAP) [5]. However, these rules are not absolute. MHC-II can also present endogenous antigens [6,7] and, likewise, there are many examples of exogenous antigens being presented by MHC-I molecules. The latter process is called cross-presentation (XP) to distinguish it from the canonical MHC-I presentation of endogenous antigens. Although XP has been a described phenomenon for over four decades [8], its molecular underpinnings have remained controversial and only recently have they begun to be uncovered in cDCs.

cDCs can be broadly divided into two subsets, known as cDC type 1 (cDC1) and type 2 (cDC2). While the identification of these cells can differ somewhat between mice and humans, cDC1s generally express markers such as XCR1, CD8α, CD24, DNGR-1 (CLEC9A), or CD103 while cDC2s often express CD11b and CD172a (SIRPα). The induction of cytotoxic CD8$^+$ T cells against viruses and tumours largely depends on cDC1s [9,10], which have been argued to possess a superior capacity for XP compared to other antigen-presenting cells (APCs) [10,11]. What exactly is meant by "superior" is often unclear. In many instances, it describes the observation that mouse cDC1 are better able than other APCs at inducing CD8$^+$ T cell responses to model antigens, including ones contained within dead cells [12]. Dead cells are arguably one of the most relevant and valuable sources of antigens encountered by cDC1s in vivo as tissue damage is intimately associated with, and often a consequence of, infection, tumour growth or injury [13,14]. Indeed, irrespective of XP, cDC1s are particularly adept at internalising material from diverse types of dead cells, including tumour cells or dying B cells, both in vivo and in vitro, in experiments comparing them to cDC2s [15,16]. Putting these two observations together, one can postulate that there likely exist mechanisms possessed by cDC1 to sense dead cells and retrieve antigenic information present in cell corpses. In this regard,

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https://doi.org/10.1016/j.smim.2023.101726
Received 28 November 2022; Received in revised form 26 January 2023; Accepted 27 January 2023
Available online 7 February 2023

recent work from our lab and others indicates that cDC1 express a receptor called DNGR-1 (also known as CLEC9A) that has a dedicated role in XP of antigens associated with dead cells. Here, we review how DNGR-1 facilitates XP of dead cell-associated antigens and discuss implications for initiation of cytotoxic $CD8^+$ T cell immunity to viruses and tumours.

2. Defining cross-presentation

XP refers specifically to the display of MHC-I loaded with peptides derived from exogenous proteins to $CD8^+$ T cells. It should be distinguished from closely related but distinct biological processes, in particular cross-priming, cross-tolerance, and cross-dressing. Cross-priming refers to a productive $CD8^+$ effector T cell response elicited by XP [17]. Cross-priming occurs in secondary-lymphoid organs and is especially relevant for eliciting anti-microbial and anti-tumour responses [18,19]. Cross-tolerance, on the other hand, involves the deletion or silencing of antigen-specific $CD8^+$ T cells in the thymus or periphery by cDCs or other APCs cross-presenting exogenous self-, dietary- or commensal-derived antigens on MHC-I molecules [20,21]. Lastly, cross-dressing involves the transfer of pre-processed peptide-MHC-I complexes from one donor cell onto an APC [22]. In this review, we focus on XP via DNGR-1 and its function in cDC1-dependent cross-priming.

Two broad models have been proposed for XP, namely the phagosome[2]-to-cytosol (P2C) (also called the cytosolic) pathway and the vacuolar pathway [23]. The vacuolar pathway posits that following exogenous material uptake into phagosomes of APCs, luminal proteases, particularly cathepsin S, break down cargo antigens into peptides [23–25]. These peptides are then loaded directly onto MHC-I within the phagosome in a process analogous to MHC-II antigen presentation [5,25]. Consistent with the notion that antigens remain enclosed within phagosomes and do not access the cytosol, the vacuolar pathway is insensitive to proteosome inhibitors yet sensitive to lysosomal protease and acidification inhibitors [23]. Although it may be important in some contexts and in some cell types, cross-priming against particulate and cell-associated antigens is only modestly affected in cathepsin S-deficient mice [26]. In the P2C pathway, phagocytosed antigens gain access to the cytosol and are then proteolytically degraded by the proteasome into peptides, which are loaded onto nascent MHC-I molecules via TAP as in the endogenous MHC-I antigen presentation pathway. Consequently, inhibition of the proteosome attenuates P2C-mediated XP [27]. The relative contribution of the vacuolar or P2C pathways to XP by cDC1s remains to be fully elucidated. In this review, we focus on evidence that DNGR-1 signalling induces XP of dead cell-associated antigens through the P2C pathway.

3. DNGR-1 expression and ligand recognition

DNGR-1 is a type II transmembrane protein belonging to group V of the C-type lectin receptor (CLR) family. It is a homodimer stabilised by a disulphide bond (Fig. 1 A). Each monomer bears an extracellular C-type lectin-like domain (CTLD) linked by a neck region to a transmembrane domain and followed by a short cytoplasmic signalling tail [28–31]. During cDC differentiation, DNGR-1 is first expressed on conventional DC progenitors (CDPs) that arise in the bone marrow and subsequently differentiate into DNGR-1^+ pre-DCs, which egress into the blood to populate tissues and give rise to differentiated cDC1 and cDC2 subsets [32,33]. DNGR-1 is highly expressed on cDC1 but not cDC2 and acts as a faithful marker of cDC1 across tissues of both humans and mice [34–36]. Its expression must therefore be upregulated during cDC1 differentiation and extinguished during cDC2 differentiation, but the responsible gene regulatory elements remain unidentified. In mice but not in humans, DNGR-1 is additionally expressed at low levels by plasmacytoid cells [28,30].

DNGR-1 expression equips cDC1 with the ability to detect dead cells. It directly binds via its CTLD to filamentous-actin (F-actin) that is exposed on cell corpses following loss of plasma membrane integrity [37–39] (Fig. 1 A). F-actin binding can take place at the cell surface but, as DNGR-1 is an endocytic receptor, recognition can also happen within an intracellular vesicle after dead cell uptake by cDC1s. F-actin is seldom "naked" and, in the cytoplasm of cells, there are around 90 different proteins that can decorate actin filaments. The F-actin-associated motor protein myosin-II potentiates F-actin triggering of DNGR-1 signalling by cross-linking F-actin in an architecture that maximises dimeric DNGR-1 binding across filaments [40]. Because F-actin (and myosin-II) are exclusively intracellular, ubiquitously expressed, and highly conserved across tissues and eukaryotic cells, exposure acts as a universal sign of cell death and tissue damage to be sensed by DNGR-1. Notably DNGR-1 binding to F-actin is negatively regulated by secreted gelsolin (sGSN), a protein that can be made by most cell types and circulates at high levels in plasma [41]. sGSN competes with DNGR-1 for F-actin binding, and thereby acts as a natural barrier to DNGR-1 dependent recognition and XP [42] (Fig. 2).

4. DNGR-1 signalling in cDC1

The tail of each DNGR-1 monomer contains a single tyrosine-based motif called a hemITAM (YxxL/I) as it resembles half of the canonical immunoreceptor tyrosine-based activation motif domain (ITAM; YxxL/$Ix_{(6-8)}$YxxL/I) found on many other immune receptors (Fig. 1 A). As for ITAMs, phosphorylation of the DNGR-1 hemITAM tyrosine (residue 7; Y7) by Src family kinases causes recruitment and activation of spleen tyrosine kinase (SYK) [31,43] (Fig. 1 A). This event is essential for the ability of DNGR-1 to promote XP as deleting or inhibiting SYK in DCs attenuates cross-priming of $CD8^+$ T cells to dead-cell associated antigen [31,44]. Although DNGR-1-SYK signalling facilitates XP (see below), it does not result in cDC1 activation nor in marked changes in cDC1 gene expression [45] (Fig. 1 A). This points to differences in hemITAM signalling between DNGR-1 and related receptors such as Dectin-1, which couple to NF-κB and MAPK pathways to induce myeloid cell activation and production of pro-inflammatory mediators [46]. The residues immediately upstream of the tyrosine in the hemITAM appear responsible for the lack of prototypical activatory activity of DNGR-1 [45], perhaps by affecting the spatiotemporal dynamics of SYK phosphorylation, although this has yet to be tested.

Under conditions of low affinity or monovalent ligand binding, a hemITAM may behave as an inhibitory motif through the recruitment of phosphates. In this regard, DNGR-1 has been shown to activate the phosphatase SHP-1 to dampen pro-inflammatory signalling by heterologous SYK-coupled receptors, including Dectin-1 [47]. Whether SHP-1 is directly recruited to the DNGR-1 receptor complex to regulate DNGR-1-induced XP has not been reported although a recent study reported that conditional loss of SHP-1 in CD11c-expressing cells, which include cDCs, led to enhanced cross-priming of $CD8^+$ T cells to *Leishmania*-derived antigens in vivo and increased XP in vitro [48]. Ubiquitination has also recently been described to regulate DNGR-1 activity. The E3 ubiquitin ligase RNF41 was found to ubiquitinate the extracellular domain of DNGR-1 with K48-linked ubiquitin chains that led to targeted degradation of the receptor [49]. Silencing of RNF41 caused an increase in both MHC-I and MHC-II presentation of dead cell-associated antigens in vitro [49]. Additional E3 ubiquitin ligases likely modulate DNGR-1 signalling and regulate XP (C.M.H. and C.R.S., unpublished observations).

[2] In this review we use the term phagosome generically to refer to a vesicle containing internalised dead cell material. In reality, such vesicles remain poorly characterised and can include endosomes and macropinosomes.

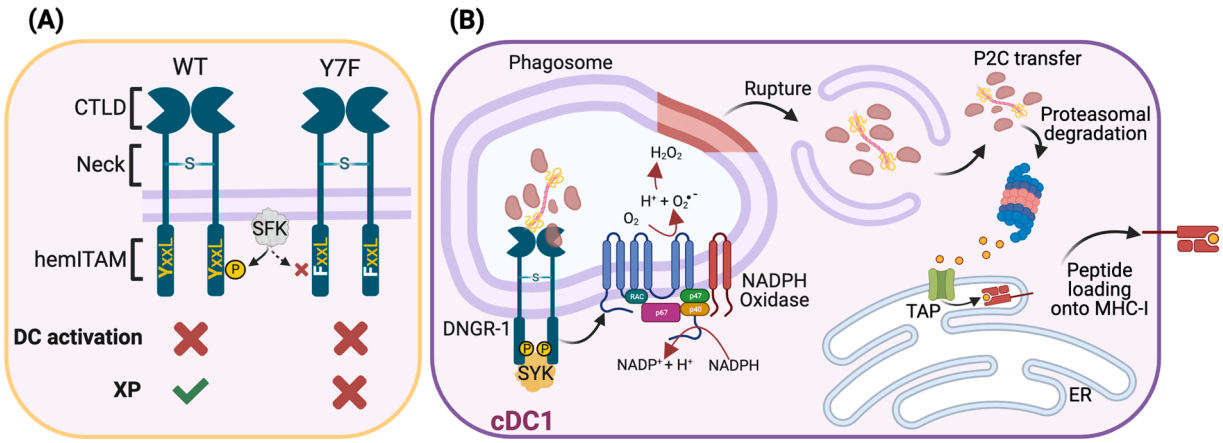

Fig. 1. DNGR-1 signalling promotes damage to phagosomal membrane and P2C. (A) DNGR-1 is a homodimer stabilised by a disulphide bond in its neck region. Binding of DNGR-1 via its CTLD domain to exposed F-actin on dead cells leads to Src family kinase (SFK)-dependent hemITAM phosphorylation and SYK activation to regulate XP but not canonical cell activation. The tyrosine 7 residue located within the hemITAM of DNGR-1 is critical for signalling. (B) DNGR-1-SYK signalling induces NADPH oxidase activation and localised ROS production, causing lipid peroxidation and membrane damage. A fraction of damaged phagosomes ruptures and releases cargo into the cytosol, where exogenous antigen can now access the endogenous MHC-I antigen presentation pathway.

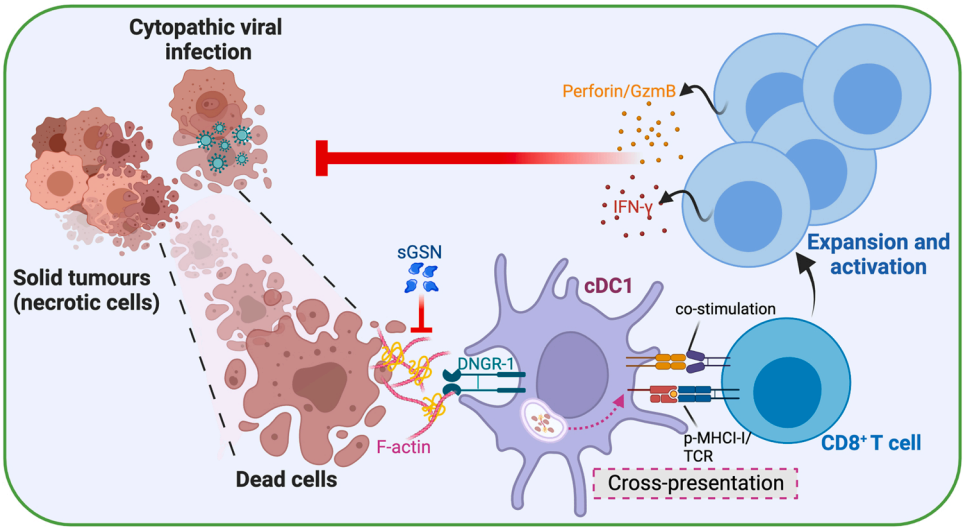

Fig. 2. Cross-presentation of tumour and viral antigens mediated by DNGR-1. Dead cells in solid tumours or cells infected with cytopathic viruses are sampled by cDC1 as they expose F-actin. DNGR-1 binds F-actin and facilitates antigen extraction from dead cell-associated material for cross-presentation. In combination with signals that increase co-stimulation on cDC1, this promotes cross-priming of protective antigen-specific $CD8^+$ T cells that secrete perforin, granzyme B (GzmB) and IFN-γ. DNGR-1 recognition of F-actin is antagonised by sGSN that circulates in plasma or is locally secreted by various cell types. p-MHC-I, peptide:MHC-I complex; TCR, T cell receptor.

5. Antigen uptake versus XP

Studies of XP have often been marred by a failure to distinguish antigen uptake from subsequent processing and loading of such antigen on MHC-I. Undoubtedly, deficit in molecules that mediate uptake of extracellular antigens by APCs also impact XP. Studies claiming to identify molecules necessary for XP must therefore differentiate between a role in uptake of antigenic substrates and/or subsequent steps in antigen handling. Further, as XP is often measured using $CD8^+$ T cell activation as a readout, adequate controls (e.g., with limiting doses of pre-processed peptide antigens) are essential to prove that a given molecule putatively involved in XP is not broadly affecting APC-$CD8^+$ T cell interactions.

Although DNGR-1 can function as an uptake receptor for latex beads coated with F-actin and myosin-II [40,43], it is dispensable for the internalisation of dead cell debris by cDC1 [31]. These observations indicate that cDC1 express additional receptors to sense and internalise dead cell remnants, which may also contribute to XP of dead cell-associated antigens [50,51]. For instance, the phosphatidylserine receptor TIM-4 is highly expressed on lung-resident cDC1 and its loss compromises the ability of cDC1 to internalise tumour material and cross-prime $CD8^+$ T cell to tumour antigens [52]. However, even though DNGR-1 deficiency in cDC1 does not decrease dead cell debris uptake, absence of DNGR-1 compromises XP of dead cell-associated antigens. To formally uncouple a role of DNGR-1 in XP from any role in uptake, we fed cDC1 with fluorescent latex beads coated in F-actin and myosin II, as well as the model antigen ovalbumin (OVA). We sorted cells that had phagocytosed a single bead and tested them for OVA XP using an antigen-specific $CD8^+$ T cell readout. These experiments revealed that cDC1 bearing wild-type DNGR-1 were more efficient at XP compared to cDC1 bearing signalling deficient (Y7F) DNGR-1 or a DNGR-1 incapable of binding F-actin (double mutant W155A, W250A) [43]. These results indicate that DNGR-1 has a non-redundant role in XP post-uptake of antigenic cargo, which is distinct from its (redundant) role as a dead cell debris uptake receptor.

6. DNGR-1 signalling promotes rupture of phagosomes

The post-uptake role of DNGR-1 in favouring XP has been the subject of investigation by our lab and others. In experiments on intracellular routing, DNGR-1 was shown to co-localise with phagocytosed dead cell material in $RAB5^+/MHC\text{-}I^+/LAMP1^-$ early non-degradative

compartments, in which intact antigens are thought to be preserved for XP or presentation to B cells [43,45,53]. However, later experiments indicated that DNGR-1 was dispensable for the formation of those compartments and that its major function in XP appears to be not in cargo routing but in promoting P2C [43].

P2C transfer of exogenous proteins has been documented using various methods, including extracellular delivery of cytochrome-c that induces apoptosis [54], extracellular delivery of ribosomal toxins that block translation [27], and extracellular delivery of proteins that are subsequently found in the cytosolic fraction of cells [55,56]. The precise mechanisms by which such proteins gain access to the cytosol has been the focus of much attention, with two main P2C scenarios posited. The first involves the selective translocation of polypeptides across phagosome membranes and is associated with the ER-associated degradation pathway, as reviewed recently [23]. The second scenario involves the non-selective leakage of phagosome contents as first articulated in the "indigestion" hypothesis, which suggested that a subset of phagosomes can rupture and release their content into the cytosol in a stochastic manner [57,58]. Notably, a connection between vacuolar compartment rupture and propensity to elicit protective MHC-I-restricted $CD8^+$ T cells responses has been noted during infection with *Mycobacterium tuberculosis* [59,60] and *Listeria monocytogenes* [59], suggesting physiological relevance for indigestion in cross-priming.

Phagosomal rupture may be favoured by reactive oxygen species (ROS) [61,62]. This requires the NADPH oxidase, which is active in phagosomes and generates superoxide (O_2^-) that reacts with a hydrogen ion (H^+) to form hydrogen peroxide (H_2O_2) (Fig. 1B). The NADPH catalytic subunit NOX2 (encoded by the gene *Cybb* in mice) is the predominant form expressed by cDCs and has been previously implicated in XP. NOX2 was described to facilitate a decreased rate of phagosomal acidification that caused delays in antigen degradation and resulted in the preservation of antigen cargo for subsequent XP [63,64]. However, DNGR-1 signalling via SYK causes acute activation of the NADPH oxidase but does not impact phagosomal acidification [43]. Instead it induces localised phagosomal membrane damage that can result in ruptures or "ulcers" in a small subset of the vesicles. These ruptured phagosomes permit transfer of phagosomal cargo into the cytosol and facilitate XP that follows in a proteasome-dependent manner [43] (Fig. 1B). The details of how NADPH-dependent ROS within the phagosome leads to phagosomal rupture remain incomplete. ROS-dependent oxidation of lipids is a likely culprit as oxidised lipids alter the physical properties of lipid bilayers, increasing permeability, promoting deformation, and perturbing ion transport [65,66]. Of note, lipid peroxidation of membranes has previously been reported to favour antigen release from endosomes and promote XP [61,62,67].

Ectopic expression of DNGR-1 can induce phagosomal membrane rupture and XP in heterologous APC such as macrophages or even non-immune cells, such as HEK293T, which also express SYK and NADPH oxidase components [43]. This is in line with a previous report that HEK293T can be rendered competent for XP of antigen-immune complexes upon expression of CD32/FcγRIIA receptor [68]. These data indicate that the phagosomal rupture P2C and XP pathway is functional in cells other than cDC1s but requires specialised receptors to activate it. Like DNGR-1, FcγRIIA also signals via SYK and so one may speculate that additional receptors that couple to SYK signalling also promote phagosomal damage, concordant with an early report that implicated ITAM signalling in XP of particulate antigens [69]. Having said that, not all SYK-coupled receptors are effective at inducing phagosomal rupture and P2C. For example, Dectin-1-SYK signalling induces only limited phagosomal rupture compared to DNGR-1 [43]. Notably, domain swap experiments show that the intracellular tail of DNGR-1 is sufficient to allow Dectin-1 to become competent to induce phagosomal damage and XP even in HEK293T cells [43]. Thus, comparison of Dectin-1 and DNGR-1 indicates yet-to-be understood regulation of the SYK-dependent pathway that leads to phagosomal damage.

DNGR-1-induced phagosomal rupture appears to occur stochastically and is limited in extent such that at any given time, only a subset of phagosomes is damaged [43]. Additionally, DNGR-1 remains circumscribed to early LAMP-1^- non-degradative phagosomes and does not cause lysosomal rupture. This may represent a mechanism to limit the cell toxicity that would be expected from a synchronised and systemic rupturing of late phagosomes and the associated release of destructive lysosomal proteases into the cytoplasm. Following DNGR-1 signalling, cytosolic sensors of membrane damage, including galectin-3 and -8, accumulate on the ruptured phagosomes through binding to exposed glycans present on the intraluminal side of the phagosomal membrane [43]. Galectins coordinate programs of repair, removal, or replacement of damaged lysosomal and vesicle compartments through the engagement of the autophagic machinery and the ESCRT-III repair pathway [70–73]. Whether rupturing phagosomes can undergo repair via ESCRT-III or are targeted by the autophagic machinery is unclear. A recent report demonstrated that shRNA silencing of the ESCRT-III components, CHMP4B and CHMP2A, in the cDC1 cell line MutuDC1940 led to increased P2C transfer of antigen and increased the capacity of the cells to XP soluble antigens and cross-prime $CD8^+$ T cells [74]. Thus, membrane repair may mitigate phagosomal damage and further limit large scale rupture.

An intriguing consequence of releasing dead cell material from phagosomes into the cytosol of cDC1s is that the process may not only ferry protein antigens for XP but also agonists for cytosolic pattern recognition receptors (PRR). The cytoplasm of cDCs contain a variety of PRRs that sense the presence of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Following phagocytosis of tumour-derived material, a fraction of tumour DNA is transferred into the cytosol of cDCs or macrophages by an undefined mechanism [75–77]. This cytosolic tumour DNA is sensed by cyclic GMP-AMP synthase (cGAS) to promote STING-IRF3-dependent type I IFN production, which feeds back via the type I IFN receptor to amplify cDC activation, antigen presentation, and cross-priming of $CD8^+$ T cells during anti-tumour immunity [75,78]. Similarly, inflammatory oxidised phospholipids (oxPAPC) generated during cell death have been reported to trigger cytosolic caspase-11 and subsequently lead to IL-1β secretion by cDCs [79]. oxPAPC is endocytosed via the TLR4 adaptor CD14, yet precisely how oxPAPC subsequently gains access to cytosolic caspase-11 is unclear [80]. Intriguingly, CD14 promotes endocytosis through a SYK-PLCγ2 pathway [81] but whether this can trigger SYK-dependent rupture of endocytic compartments is presently undefined. A likely consequence of release of PAMPs/DAMPs present within dead cell debris into the cytosol could be indirect cDC1 activation by cytosolic PRR signalling. This could compensate for the inability of DNGR-1 signalling to directly promote cDC activation and help potentiate cross-priming of $CD8^+$ T cells to dead cell-associated antigens.

7. DNGR-1 in anti-viral immunity

When not directly infected by viruses, cDCs must employ XP of extracellular viral antigens to initiate a virus-specific $CD8^+$ T cell response. A comprehensive study of the function of DNGR-1 in anti-viral immunity was performed by infecting mice with attenuated Western Reserve vaccinia virus strains [44]. DNGR-1-deficient mice displayed reduced generation of vaccinia virus-specific $CD8^+$ T cells, impaired resolution of the viral lesion and had higher viral loads in the late phase of the infection compared to control mice [44]. DNGR-1-dependence was particularly marked when using a mutant vaccinia virus strain that induces apoptosis more readily in infected cells, which limits direct MHC-I presentation from infected DCs and thereby favours dependence of the $CD8^+$ T cell response on cross-priming [44]. Lastly, when DNGR-1-deficient mice were immunised with the modified vaccinia Ankara (MVA) attenuated vaccine (currently used against monkeypox virus infection in humans) and then challenged with wild type vaccinia virus, they displayed impaired secondary virus-specific $CD8^+$ T cell responses, high viral load, and delayed resolution of viral lesions [44].

Studies with other viruses also suggest a role for DNGR-1 in $CD8^+$ T cell priming (Fig. 2). DNGR-deficient mice mount reduced $CD8^+$ T cell responses during respiratory infection with herpes simplex virus-1 [45] and influenza A virus (IAV) [82], although not following infection with respiratory syncytial virus (RSV) [83]. Some of the differences in degree of DNGR-1-dependence in different virus infection models may reflect the relative contributions of direct versus cross-priming, as well as DNGR-1-independent mechanisms of XP. A follow-up study with the vaccinia virus and IAV models showed that after resolution of experimental infection, DNGR-1-deficient mice displayed reduced virus-specific $CD8^+$ T tissue-resident memory (T_{RM}) cells but not circulating memory $CD8^+$ T cells in lymphoid tissues [82]. T_{RM} cells provide robust immunity to reinfection or secondary immune challenge and may help to curb challenging pathogens, such as HIV, *Plasmodium* parasites, and others [84]. The finding that DNGR-1 plays a non-redundant role in $CD8^+$ T_{RM} cell generation suggests that favouring XP may be useful for next-generation vaccines to elicit protective immunity.

Antigen targeting to DNGR-1 can be used to promote cytotoxic $CD8^+$ T cell responses. Viral antigens coupled to monoclonal antibodies against DNGR-1 successfully targeted human cDC1 and elicited $CD8^+$ T cell recall responses to cytomegalovirus from human peripheral blood mononuclear cells and induced them in humanised mice [85]. DNGR-1-directed antigen delivery also facilitates $CD4^+$ T cell responses and humoral antibody immunity dependent on T follicular helper (T_{FH}) cells although the mechanism remains unclear [86,87]. Thus, antigen delivery to DNGR-1 could be used as a strategy for vaccine design in infectious disease, favouring XP for cytotoxic $CD8^+$ T cell immunity and other antigen processing mechanisms for helper $CD4^+$ T cell and antibody responses.

8. DNGR-1 in anti-tumour immunity

Tumours often have areas of necrosis resulting from inadequate vascularisation, heightened levels of hypoxia and nutrient deprivation. Necrotic cancer cells in those areas or elsewhere in the tumour represent a relevant source of tumour-associated antigens (TAAs) [88,89]. cDC1 are non-redundant for anti-tumour immunity in many mouse transplantable tumour models [90–94] and cDC1 abundance within human tumours correlates with favourable outcome and positive response to immune checkpoint blockade therapy [9,95]. Thus, DNGR-1-mediated XP of dead tumour cells would be predicted to contribute positively to anti-tumour immunity. Surprisingly, however, DNGR-1-deficient mice do not display overt deficiencies in controlling immunogenic transplantable tumours. In fact, paradoxically, a recent study suggested a role for DNGR-1 in limiting anti-tumour immunity in a setting in which tumour cells overexpress the DC growth factor Flt3L [96]. It is possible that necrotic death in rapidly growing transplantable tumours may happen too late for DNGR-1 detection to be meaningful. It is also possible that the "visibility" of such dying cells to cDC1 may be limited, particularly in a setting in which DNGR-1 recognition is being antagonised by the highly abundant actin scavenging protein, secreted gelsolin (sGSN). Indeed, mice lacking sGSN displayed reduced growth of some transplantable tumours but only in DNGR-1 sufficient mice, as those deficient in both sGSN and DNGR-1 did not exhibit this increased tumour growth resistance [42] (Fig. 2). A follow-up study further revealed that sGSN also dampens the response to anti-tumour therapies, including chemotherapy and radiotherapy, only in DNGR-1 sufficient animals [97]. Both studies reveal a context-specific role for DNGR-1 in eliciting anti-tumour responses in pre-clinical transplantable tumour models in the setting of sGSN absence. Extending to humans, analysis of The Cancer Genome Atlas datasets showed that in human liver hepatocellular carcinoma, head and neck squamous cell carcinoma, or stomach adenocarcinoma, low expression of *sGSN* transcripts in cancer biopsies was associated with higher patient overall survival [42]. This was particularly marked for patients displaying mutations in one or more F-actin binding proteins, suggesting that the latter can act as tumour antigens when made immunologically visible via the DNGR-1-dependent XP pathway [42].

9. Concluding remarks

As reviewed above, DNGR-1 has a dedicated role in inducing XP of dead-cell associated antigens. A few points are worth noting in conclusion.

DNGR-1 signalling induces P2C by favouring rupture of phagosomes, consistent with the "indigestion" model. The fact that this is a property of the receptor rather than the cDC1 from which it originates indicates that XP can be an inducible process, triggered by receptors that detect antigenic cargo that may be present in dead cell corpses. This is not incompatible with the notion that cDC1 possess cell biological specialisations that increase the efficiency of P2C and XP compared to other APCs, such as slowly maturing phagosomes that retain undegraded dead cell cargo or reduced endosomal membrane repair. Nevertheless, it suggests that many cell types possess the machinery for P2C and can be coaxed into more efficient XP by receptors and signals that augment phagosomal rupture.

Although DNGR-1 contributes to XP of dead cell-associated antigens, the receptor is not involved in XP of other antigenic modalities such as soluble proteins, antigen-coated latex beads, bacteria, etc. In fact, even XP of dead cell-associated antigens is not fully ablated in *Clec9a*$^{-/-}$ mice. These observations suggest the existence of additional receptors that promote XP, as previously noted [50,51,68]. To what extent such receptors also signal via SYK and activate the NADPH oxidase to promote endosomal or phagosomal rupture or engage distinct pathways of XP (involving P2C or not) remains to be uncovered. And, of course, non-receptor mediated processes (e.g., macropinocytosis [55]) can also contribute to XP.

DNGR-1-induced P2C is finely balanced by negative regulation of DNGR-1 recognition and signalling. sGSN competes with DNGR-1 for F-actin binding, limiting engagement of the receptor. This may help keep in check detrimental $CD8^+$ T cell responses to cytoskeletal proteins [98], although the extent to which sGSN contributes as a barrier to autoimmunity remains to be determined. Intracellular signalling is also highly regulated as denoted by the differences in outcome of DNGR-1 versus Dectin-1 engagement at the level of P2C and cell activation. A more complete understanding of the positive and negative regulators of DNGR-1 signalling may provide further insights into how this receptor regulates XP of dead cell-associated antigens.

A final important facet of DNGR-1 is that it does not signal for DC activation. The selective role of DNGR-1 in XP reveals a class of innate immune receptors that can regulate the "antigenicity" of dead cells. This distinguishes DNGR-1 from the types of activatory innate immune receptors predicted by the "Danger model" [99], which are meant to decode the "adjuvanticity" of dead cells. This means that additional signals emanating from dying cells are needed to activate cDC1 to ensure that XP results in cross-priming of $CD8^+$ T cells. In the absence of such signals, DNGR-1-mediated XP could in theory contribute to cross-tolerance. Whether this happens in vivo is unclear although it is worth noting that DNGR-1 recognition of exposed F-actin is likely to happen only in conditions in which many DAMPs are being released, which would tend to prevent tolerance. Besides promoting APC activation, signals from recognition of DAMPs and PAMPs can also contribute to XP [100] and may therefore synergise with DNGR-1 signalling to promote P2C. Notably, cDC1 express high levels of TLR3, which contributes to cDC1 cross-priming against virally-infected cells containing double stranded RNA [101]. Exploring the interplay between DNGR-1 and PRR signalling may therefore prove a fruitful strategy for increasing P2C, XP and cDC1 activation, thereby favouring cross-priming to promote protective $CD8^+$ T cell responses to cancer and viral infection.

Funding

Research in the Immunobiology Laboratory is supported by The Francis Crick Institute, which receives core funding from Cancer Research UK (CC2090), the UK Medical Research Council (CC2090), and the Wellcome Trust (CC2090), by an ERC Advanced Investigator grant (AdG 268670), by a Wellcome Investigator Award (WT223136/Z/21/Z), and by a prize from the Louis-Jeantet Foundation. This research was funded in whole, or in part, by the Wellcome Trust (grants CC2090 and 223136/Z/21/Z). C.M.H. was supported by a Marie Skłodowska-Curie Individual Fellowship under Horizon 2020 from the European Union (grant no. 792770). For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Declaration of Competing Interest

C.R.S. is a founder of Adendra Therapeutics and owns stock options and/or is a paid consultant for Adendra Therapeutics, Bicara Therapeutics, Montis Biosciences, Bicycle Therapeutics and Sosei Heptares. C. R.S. has an additional appointment as a Visiting Professor in the Faculty of Medicine at Imperial College London and holds honorary professorships at University College London and King's College London.

Acknowledgements

We thank Michael Buck for reviewing the manuscript and members of the Immunobiology Laboratory for helpful discussions and suggestions.

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