Phagocytosis and antigen presentation: a partnership initiated by Toll-like receptors

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ABSTRACT

Professional phagocytes play an important role in the clearance of microbial pathogens and apoptotic cells. Many receptors are involved in this process, some with signalling capabilities, some without. Increasing evidence now shows a previously unappreciated regulatory component to phagocytosis exerted by the concomitant engagement of signalling receptors. The engagement of Toll-like receptors (TLRs) during phagocytosis of microbial pathogens is the best characterised example. Here, a brief overview is presented of the findings that TLRs exert positive and phagosome autonomous control on both the kinetics and outcomes of phagosome maturation. Although phagosomes could mature in the absence of TLR signals, they did so at a slower constitutive rate. Engagement of TLRs from another phagosome or from the plasma membrane did not affect the constitutive maturation of phagosomes devoid of TLR ligands. This was also reflected in the superior ability of phagosomes carrying TLR ligands to contribute peptides to major histocompatibility complex (MHC) class II molecules. Thus, TLR control of antigen presentation favours the presentation of microbial antigens within the context of T-lymphocyte costimulatory molecule expression. This current work aims to identify whether TLRs exert similar control on the presentation of phagocytosed antigens within MHC class I molecules, a process referred to as cross-presentation.

A necessary step towards the goal of improving health and curing human disease is to expand our fundamental understanding of how immune responses are initiated and which elements exert the most dramatic impact on the immunological outcome. Dendritic cells (DCs) play a major role in inducing immunity or tolerance. The nature of the signals delivered by DCs to T cells determines T-cell activation, clonal expansion and differentiation.[1 2] Two key measures that determine the quality of the immune response are the mode of antigen internalisation and the type of signal transduction pathways triggered in DCs. We will discuss each parameter in detail and then discuss how coupling the two measures achieves optimal immune activation.

MODE OF ANTIGEN DELIVERY

An important cellular function all eukaryotic cells share is internalisation. Internalisation relies primarily on the endocytic pathway that serves cellular functions as diverse as plasma membrane homoeostasis, uptake of nutrients and growth factors and host defence. DCs have the extraordinary ability to internalise larger particulates through a distinct form of internalisation called phagocytosis.[3] In addition to phagocytosis, DCs perform different types of receptor-mediated endocytosis and macropinocytosis,[4] all of which depend on vesicular traffic to deliver internalised cargo into distinct endocytic compartments.[5 6] DCs express many different types of receptors with varying ligand specificities for proteins, oligosaccharides, nucleic acids and lipids.[7] Many of these receptors can be engaged during internalisation into DCs, but an important distinction among these receptors is that they vary greatly in their delivery of internalised cargo to different endocytic compartments.[8–11]

The intersection of endocytic pathways with major histocompatibility complex (MHC)-containing subcellular compartments critically determines the course and character of antigen presentation. Phagocytosis is a particularly effective route to deliver antigens into MHC class II rich compartments.[3] Newly synthesised MHC class II associate with invariant chain (Ii) in the endoplasmic reticulum (ER) to form a nonameric complex consisting of three $\alpha\beta$ dimers and three Ii molecules.[12 13] A targeting sequence within the Ii cytoplasmic domain targets this nonameric complex to the endocytic pathway where MHC class II can potentially encounter and bind peptides derived from internalised antigens. The association of Ii also prevents premature loading of peptides onto MHC class II.

Phagocytosis is also an efficient route to deliver exogenous antigens for cross-presentation by MHC class I.[3 14] In the classical MHC class I presentation pathway, endogenous proteins are degraded by various peptidases and the proteasome. The resulting peptides are translocated into the ER lumen by the transporter associated with Ag processing (TAP).[15] There, newly synthesised MHC class I chaperoned by calnexin associate with a large MHC class I loading complex and become loaded with TAP-transported peptides. In the case of cross-presentation, a mechanistic problem arises in delivering exogenous antigens to ER resident MHC class I, but several routes do exist.[16] Gap junctions can allow direct transfer of peptides from infected cells into the cytosol of DCs.[17]

STATUS OF DC ACTIVATION

The activation state of DCs is critical for augmenting an immune response. The signal transduction pathways that have the most remarkable impact by far on DC function are those downstream of Toll-like receptors (TLRs). TLRs recognise various structurally unrelated and evolutionarily conserved pathogen-associated molecular patterns

(PAMPs).[18 19] Signalling pathways initiated by TLRs involve the adaptor proteins myeloid differentiation factor 88 (MyD88), Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein TIRAP/MyD88-adaptor-like (MAL), TIR-domain containing adaptor protein inducing interferon (IFN)β (TRIF)/TIR-domain containing molecule 1 (TICAM-1) and TRIF-related adaptor molecule (TRAM).[19] The result is activation of mitogen activated protein kinases (MAPK) p38, ERK and JNK and transcription of nuclear factor for κB (NF-κB) and interferon regulatory factor (IRF)-responsive genes pivotal to immunity. MyD88 and TRIF/TICAM-1 are the signalling adaptors whereas TIRAP/MAL and TRAM are sorting adaptors.[20] The activation of DCs determines their ability to deliver three signals to naïve T lymphocytes:

- Signal 1 is delivered to the T-cell receptor through engagement of the cognate peptide–MHC complex. It is generally thought that all antigens internalised through various forms of endocytosis are delivered into late endosomal and lysosomal compartments where their processed peptides are automatically loaded onto MHC class II molecules.[21] However, we have recently shown, as we discuss below, that despite their presence within the right compartments, MHC class II are not receptive to peptide loading after phagocytosis unless triggered by signals from TLRs.[22]
- Signal 2 is delivered to T cells through costimulatory molecules. A critical determinant for T-cell activation is the expression of T-cell costimulatory molecules on DCs.[1 23 24] Naïve T cells are tolerised when they receive signal 1 alone and primed when they receive signal 1 along with costimulation. Expression of costimulatory molecules on DCs is induced by triggering TLR signalling pathways.[1] Therefore, the engagement of TLR signalling pathways in DCs controls two key parameters necessary for optimal T-cell activation, the presentation of peptides within MHC class II and the expression of costimulatory molecules.
- Signal 3 delivery to T cells determines their differentiation fate. Signal 3 refers to DC-derived signals that determine T-cell differentiation into a particular lineage and thus dictate the class of immune response.[24] A plethora of studies have delineated the activation and differentiation requirements of CD4 T cells into Th1 cells, Th2 cells, Th17 and regulatory T cells.[24] Importantly, many of the signals that constitute signal 3 are controlled by TLRs.[19 24 25] For example, inflammatory cytokines such as interleukin 12 (IL12), IL18 and type I IFNs produced by DCs skew CD4 T-cell differentiation into Th1 cells. For Th2 differentiation, DC expression of the Jagged family of Notch ligands, as one example, is thought to be important.[26] Th1 cells produce IFNγ and mediate the elimination of bacterial and viral infection, while Th2 cells, which produce IL4 and IL13, are involved in the response against helminth infection. Stimulation of most TLRs leads to Th1 rather than Th2 differentiation.

Our work so far has been shaped by our original hypothesis that TLR signalling is coupled to the phagosome–lysosome pathway and has an impact on its outcomes. Why would coupling between receptor signalling and endocytic pathways exist in the first place?[8–10 27–29] We will consider this question next because it is relevant to understanding why TLR signalling can exert regulatory control over phagosome maturation and MHC class II presentation and, possibly also, cross-presentation.

COUPLING INTERNALISATION WITH SIGNAL TRANSDUCTION

The same cellular machinery used for internalisation as part of a cell's normal housekeeping functions can also provide a portal of entry for microbial pathogens.[30 31] Internalisation pathways can thus directly deliver pathogens to a new intracellular niche that provides shelter from extracellular mechanisms of host defence. With this loophole in internalisation comes the need for immunosurveillance mechanisms that patrol the endocytic pathway. Indeed, immunosurveillance mechanisms that patrol the endosome–lysosome pathway are the most efficient and the best characterised.[6 32 33] We reasoned that the assignment of host defence functions to phagocytosis must have necessitated the emergence of a link during evolution between the endocytic pathway and signal transduction pathways that protects the host cell against infection. This would ensure that internalisation of cargo that is harmful to the cell, such as microbial pathogens, is coupled to the activation of signalling pathways that trigger innate immune defence functions.

The TLR signalling pathway is a prime candidate for controlling the endocytic pathway. TLRs are expressed on the plasma membrane and along the endocytic pathway, placing them at the proper locations for detecting PAMPs.[19] TLR1, 2, 4, 5 and 6 are primarily expressed on the plasma membrane. TLR2 was also shown to be present on the membranes of nascent phagosomes forming around *Saccharomyces cerevisiae*.[34] Likewise, TLR4 is also found in early endosomes.[35] Signalling from plasma membrane TLRs results in transcriptional activation of inflammatory cytokines such as IL12 and IL6. TLR3, 7, 8 and 9 are confined to late endocytic compartments and their ligands are all microbial constituents exposed after some degree of degradation. For example, TLR3 recognises double-stranded RNA, TLR7 and 8 recognise single-stranded RNA and TLR9 recognises CpG DNA motifs. TLR activation by microbial nucleic acids triggers the production of type I interferons that have potent antimicrobial activities.

TLR CONTROL OF PHAGOSOME MATURATION

We were the first to demonstrate that TLR signalling pathways, in addition to initiating transcription of cytokines and costimulatory molecules, also regulate the phagosome–lysosome endocytic pathway to enhance antigen presentation and host defence.[8 9 36] We found that TLRs control multiple aspects of phagocytosis, including internalisation and phagosome maturation, as well as functional outcomes such as antigen presentation within MHC class II.[22 36] The first observation we made was that macrophages deficient in TLR signalling were less efficient in phagocytosis of bacteria than wild-type controls.[36] Fewer bacteria were internalised in the absence of TLR signalling over time, suggesting that phagocytosis was enhanced in the concomitant presence of signals from TLRs. Electron microscopy showed that in the absence of TLR signalling, bacteria were indeed internalised but in smaller numbers. The key observation we made was that electron-dense lysosomes could dock onto phagosomes containing bacteria, but there appeared to be a block in the ability of phagosomes to fuse with these lysosomes. These results indicated a defect in phagolysosomal fusion and proper phagosome maturation in the absence of TLR signals.

We performed further studies to confirm this. When TLR signalling was engaged, an inducible mode of phagosome maturation was observed with distinct kinetics and functional consequences. Microbial pathogens like *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium*, all engaged TLRs during internalisation and were delivered to lysosomes at an inducible rate manifested by increased clearance and phagolysosomal fusion.[36] This inducible rate was evident in two situations. The first showed that maturation of bacteria

containing phagosomes proceeded at an inducible rate in the presence of TLR signalling. The second showed that phagocytosis of apoptotic cells proceeded at the same rate in the presence or absence of TLR signals. This rate was similar to the rate at which phagosomes containing bacteria matured in the absence of TLR signals. We noted that in the absence of TLR signalling, either with macrophages deficient in TLR signalling or with apoptotic cell cargo that lack TLR ligands, the kinetics of phagosome maturation proceeded similarly at the constitutive rate. We concluded that there were two modes of maturation, inducible and constitutive, with the inducible mode controlled by TLR signals.

Notably, we found that TLR control of the kinetics and function of phagosome maturation was phagosome autonomous such that within the same cell, maturation of phagosome containing apoptotic cells was not influenced by TLR signalling at the plasma membrane or at the membrane of a different phagosome containing bacteria.[36] This we found by asking whether we could increase the constitutive mode of phagosome maturation by superimposing a TLR signal. We followed maturation of phagosomes containing apoptotic cells with or without stimulation of TLR4 at the plasma membrane by lipopolysaccharide (LPS). We also followed maturation of phagosomes containing apoptotic cells with or without stimulation of TLRs from phagosomes containing bacteria. The question we asked was: can the TLR signal from these phagosomes influence the maturation of apoptotic cell-containing phagosomes? The answer was ''no''. Only phagosomes containing *E coli* matured at the inducible rate. The maturation of phagosomes containing apoptotic cells was not enhanced by the exogenous addition of LPS or simultaneous phagocytosis of *E coli*. Therefore, the TLR signal coming from phagosomes containing bacteria did not affect the maturation rate of phagosomes containing apoptotic cells. This suggested that phagosome maturation was stimulated by a TLR signal that was spatially confined and phagosome autonomous, such that only phagosomes containing cargo that engaged TLRs were subject to the inducible rate of phagosome maturation. The compartmentalised nature of the TLR signal also suggested that a distinct signalling complex might assemble along the membranes of phagosomes carrying microbial cargo. This complex may form around a TLR-activated p38 MAPK scaffold. One could envision that this TLR-initiated complex would probably endow on that phagosome a distinct TLR-based molecular signature that the DCs may use in order to discriminate the contents of that phagosome. This molecular signature could then dictate the immediate fate of the cargo and the immune responses tailored to that cargo.

TLR CONTROL OF MHC CLASS II ANTIGEN PRESENTATION

The processing of phagocytosed cargo within phagolysosomes results in the degradation of cargo proteins into smaller peptides that are assembled with MHC class II and transported to the plasma membrane for recognition by CD4 T cells with the corresponding T-cell receptor specificity. This process is an important immunological consequence of phagosome maturation. One consequence of phagosome individuality is the uncoupling of processing and presentation from TLR-induced expression of costimulatory molecules. This might play an important physiological role in tissues during infection where DCs can phagocytose both microbial pathogens and tissue apoptotic cells. In such a scenario, it is expected that MHC class II would present immunogenic peptides from both types of phagocytosed cargo to naïve CD4 T cells in the context of T-cell costimulatory molecules. Although this is ideal for the activation of T cells against microbial derived antigens, the activation of T cells against apoptotic cell derived antigens can lead to adverse autoimmune responses. The explanation offered for this dilemma was that T cells with specificities to self-antigens are deleted before the onset of infection.[37]

We wanted to simulate this process in vitro and test whether DCs were capable of discriminating the contents of their phagosomes by reading TLR-based molecular signatures on these phagosomes. We gave DCs both bacteria and apoptotic cells at the same time and we engineered these cargos such that they carried different antigens. We then sorted DCs that carried both types of cargo and added antigen-specific CD4 T cells. Then we measured their activation.[22] We found that after phagocytosis of apoptotic cells, DCs do not present apoptotic cell derived antigens to specific T cells as measured by T-cell production of IL2 and this was true whether the apoptotic cell antigen was Eα or whether it was OVA. When both apoptotic cells and *E coli* were phagocytosed at the same time, either the combination of Eα from apoptotic cells and OVA from bacteria or the other way around, OVA from apoptotic cells and Eα from bacteria, in each case only presentation of bacterial derived antigen was seen. The apoptotic cell derived antigen was not presented and this was despite the increased levels of MHC class II molecules on the cell surface. However, if we took apoptotic B cells that had previously been stimulated with a TLR ligand such as LPS, we in effect introduced a TLR ligand within the same phagosome containing apoptotic cells. Now, phagocytosis of these apoptotic LPS blasts did result in presentation of apoptotic cell derived Eα to T cells. We observed the same result using costimulation-independent T-cell hybridomas as the readout for antigen presentation.

Importantly the presentation of apoptotic cell derived antigen from apoptotic LPS-stimulated B cells was not due to the blasting status of B cells before inducing apoptosis as Eα from anti-immunoglobulin-stimulated B cells was not presented.[22] Furthermore, this presentation was dependent on the presence of a TLR ligand as it was severely impaired in the absence of TLR4 expression by the presenting DC. Increasing the number of apoptotic cells fed to DCs did not result in presentation of apoptotic cell derived antigens either. In contrast, there was a dose-dependent increase in the presentation of antigen derived from apoptotic LPS blasts. The TLR ligand needed to be compartmentalised within the same phagosome as the apoptotic cell since the exogenous addition of LPS does not result in appreciable presentation of the apoptotic cell derived antigen either.

In summary, we showed that using the mechanism of TLR-dependent selection of antigens, only antigenic peptides derived from phagosomes that engaged TLR signalling were presented by MHC class II on the plasma membrane.[22] Apoptotic cells did not engage TLRs and presentation of their antigens was not detected, regardless of the engagement of TLR signalling in the same cell at the plasma membrane by soluble TLR ligands or from phagosomes containing bacteria.

We next used antigen-conjugated microspheres that allowed us to directly control the size of phagocytosed cargo, the amount and form of antigen phagocytosed by DCs and the presence or absence of TLR ligand on the cargo. We conjugated LPS-free hen egg lysosyme (HEL) to the surface of inert microspheres and adsorbed half of these microspheres with LPS, leaving the other half untreated.[22] Only HEL-LPS-conjugated microspheres could engage TLR signalling as shown by

their ability to induce IL6 production by DCs. We gave HEL-conjugated microspheres to DCs in three combinations: Either HEL-conjugated microspheres alone, HEL microspheres and exogenous LPS at the same time, or HEL/LPS-conjugated microspheres. We then monitored the activation of HEL-specific 3A9 T-cell receptor transgenic CD4 T cells by their production of IL2 in response to different doses of microspheres. We found that (*a*) only HEL derived from HEL/LPS microspheres that engaged TLR signalling was efficiently presented to and activated 3A9 CD4 T cells in a dose-dependent manner; (*b*) there was no MHC class II presentation of HEL derived from HEL-conjugated microspheres and (*c*) the simultaneous addition of exogenous LPS again did not result in appreciable MHC class II presentation. Collectively, there were two results here: first, MHC class II presentation of phagocytosed cargo was controlled by TLR signalling and second, this control was compartmentalised in that the engagement of TLRs at the plasma membrane by the addition of soluble LPS did not enhance presentation, but rather it was necessary for the TLR ligand to be present within the same phagosome as the apoptotic cell.

To observe directly phagosome autonomous formation of immunogenic peptide:MHC class II complexes at the single-cell level, we monitored the intracellular formation of the specific HEL presented by the MHC class II molecule using a specific monoclonal antibody called C4H3. By using two different sizes of HEL-conjugated microspheres differentially adsorbed with LPS, we were able to discriminate HEL from HEL/LPS phagosomes by confocal microscopy.[22] Cholera toxin B subunit staining before permeabilisation allowed differentiation between intracellular and surface C4H3. We consistently found preferential formation of HEL peptide MHC class II complexes intracellularly on HEL/LPS phagosomes and not HEL phagosomes. We subsequently showed that TLR control of antigen presentation by MHC class II was at the level of cleavage of Ii, a chaperone that protects the peptide binding groove of newly synthesised MHC class II molecules.[22] Ii was specifically cleaved within phagosomes containing TLR ligands and not within those devoid of TLR ligands. This was irrespective of the exogenous addition of LPS to DCs.

Based on these findings, we proposed a model where upon simultaneous phagocytosis of microbial and apoptotic cells, phagosomes containing microbial cargo that engage TLRs are favoured for MHC class II presentation and do not influence the fate of apoptotic self-cargo within other physically distinct phagosomes.[10] When this mechanism is used, DCs can distinguish between different types of phagocytosed cargo based on the presence or absence of TLR ligands and as a consequence classify different sources of antigens as self or non-self at the subcellular level. The result is compartmentalised generation of peptide–MHC class II complexes where the contents of phagosomes derived from microbial pathogens are preferentially presented in the context of costimulation. This TLR-based mechanism of discriminating phagosomal contents would ensure that upon simultaneous phagocytosis of self and non-self, antigens derived from self are excluded from the pool of MHC class II transported to the plasma membrane upon concomitant TLR engagement by microbial pathogens. Thus, one important functional consequence of compartmentalised TLR control of phagosome maturation in DCs is selection of antigenic cargo for MHC class II presentation, which together with TLR-induced expression of costimulatory signals contributes to peripheral self/non-self discrimination.

SUBSEQUENT AND FUTURE WORK

One would expect that other pattern recognition receptors engage a similar pathway of inducible phagosome maturation. Indeed, macrophages derived from mice deficient in Syk tyrosine kinase or all three members of the Src-family kinases, Hck, Fgr and Lyn, which are activated "downstream" of FcR signalling, exhibit a slower rate of phagocytosis of IgG-coated sheep erythrocytes.[38 39] However, one study examining the phagolysosomal fusion of beads coated with either mannose or IgG found no differences in the rate of phagolysosomal fusion of phagosomes containing mannose- or IgG-coated beads and phagosomes containing mannose- or IgG-coated beads in addition to ligands that stimulate TLRs.[40] The authors' interpretation of such results was that TLRs do not regulate phagosome maturation.[40] However, given the abundant reports supporting prominent regulation of phagocytosis by TLRs,[22 36 41–46] we favour an alternative interpretation: engagement of phagocytic inflammatory receptors in this study, (the macrophage mannose receptor by mannose and the FcR by IgG) probably already induces maximal kinetics of phagolysosomal fusion, which cannot be increased by addition of a TLR ligand. Out of space consideration, we refer to previous reports[10 47 48] for further discussions on the topic.

A recent study from the laboratory of Douglas Green reproduced our previous findings on TLR-mediated rapid acidification of phagosomes, and also provided further evidence that TLRs mediate recruitment of the autophagy machinery to phagosomes, a recruitment that turns out to be essential for allowing the rapid kinetics of phagosome maturation we had originally described.[36 49] Consistent with our previous studies,[22 36] and contradictory to those described by Yates and Russell,[40] the authors showed that in contrast to the slow and limited maturation of phagosomes carrying inert beads, coupling beads to a TLR ligand induced more rapid and extensive phagosome acidification.[49] Remarkably, increased phagosome maturation was inhibited by knockdown of the autophagy pathway protein ATG5, suggesting that TLR-induced recruitment of classical autophagy pathway proteins to phagosomes promoted their fusion with lysosomes.[49]

Recent follow-up studies from our laboratory as well as others have demonstrated that TLR engagement has dramatic effects on the efficiency of antigen processing and presentation.[22 41–44] The laboratory of Colin Watts showed that macropinocytosis was also controlled by TLRs.[42] TLR ligands were shown to acutely stimulate antigen macropinocytosis, leading to enhanced presentation by MHC class II and MHC class I. Also consistent with our studies, the laboratory of Alan Sher established that presentation by DCs of *Toxoplasma gondii* profilin, a TLR11 protein ligand, required both TLR signalling and MHC class II expression by the same DCs, supporting a major influence of TLR recognition in antigen presentation by DCs in vivo. In addition, Marcus Groettrup and colleagues found that the co-encapsulation of TLR ligands and antigen onto biodegradable microspheres as compared with co-injection of antigen with TLR ligands, was superior in priming antigen-specific cytotoxic T cells in vaccinated mice.[50] New studies from Laurence Zitvogel's laboratory demonstrated that activation of tumour antigen-specific T-cell immunity requires secretion of the high-mobility-group box 1 (HMGB1) protein by necrotic tumour cells and the action of HMGB1 on TLR4 expressed by DCs.[41] DCs required signalling through TLR4 and MyD88 for efficient processing and cross-presentation of antigen from dying tumour cells. Patients with breast cancer carrying a *TLR4* loss-of-function allele relapsed more quickly after radiotherapy/

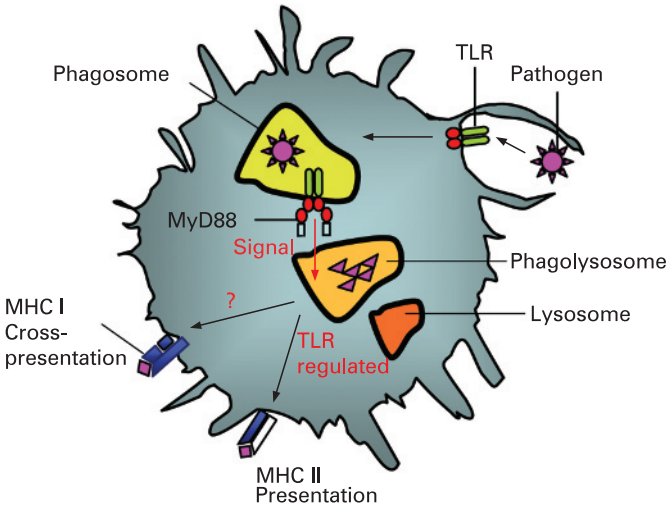

Figure 1 A schematic diagram of phagosome maturation and Toll-like receptor (TLR) signals. A dendritic cell in blue is depicted in the process of phagocytosis of a microbial pathogen, which engages TLRs during internalisation. Once inside the cell, the pathogen is enclosed within a newly formed phagosome which undergoes a series of sequential fusion events with the endocytic pathway in a process called phagosome maturation culminating in phagolysosomal fusion and terminal delivery of phagocytic cargo to lysosomes. The engagement of TLRs during phagocytosis regulates phagosome maturation, which in turn leads to two outcomes: major histocompatibility complex (MHC) class II presentation and cross-presentation. We have shown that one of these outcomes, MHC class II presentation is regulated by TLR signals. The question we ask now is whether cross-presentation is also regulated by TLRs.

chemotherapy than those carrying the normal *TLR4* allele. These results further delineate a clinically relevant immunoadjuvant pathway triggered by tumour cell death and mediated by TLR4. More recently, Christian Kurts and colleagues demonstrated that efficient cross-presentation of soluble antigen required TLR-MyD88 signalling and appeared biased towards antigens containing microbial molecular patterns.[51]

We have shown that one consequence of communication between TLR signalling pathways and the phagosome–lysosome pathway is TLR control of phagosome maturation and MHC class II presentation.[22] Whether cross-presentation of phagocytosed antigens is also dependent on TLR signalling is not clear (fig 1). In the absence of TLR signalling, cross-presentation may occur constitutively to ensure that CD8 T cells with autoreactivities to endogenous cellular proteins have the opportunity to encounter them in the absence of inflammation and become tolerant. Alternatively, it is possible that true to the proposed physiological role of cross-presentation in antiviral immunity,[14 52] only infected apoptotic cells may successfully be cross-presented, where viral nucleic acids within these cells could trigger TLRs. Conflicting studies exist showing either outcome.[53–65] The general consensus is that constitutive cross-presentation does occur at steady state.[66] A direct comparison, however, in the presence or absence of TLR signals has not been made. Our current work is aimed at identifying whether TLRs also control cross-presentation of phagocytosed antigens within MHC class I molecules.

Competing interests: None.

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Notes