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No life without death—apoptosis as prerequisite for T cell activation

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The orchestrated death of infected cells is key to our understanding of CD8 T cell activation against pathogens. Most intracellular bacteria including *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis, remain enclosed in phagosomes of infected macrophages. CD8 T cells play a critical role in defense of infection and recognize antigens originating from the cytosol presented by MHC-I molecules. Since mycobacteria do not gain access to the cytosolic MHC-I presentation pathway, the fundamental question as to how CD8 T cells encounter mycobacterial antigens remains to be solved. In this review, we focus on solutions for this enigma and describe the detour pathway of T cell activation. Mycobacteria induce cell death of infected macrophages which thereby leave a last message by releasing apoptotic vesicles. Subsequently, these antigen-containing entities are engulfed by dendritic cells which process the mycobacterial cargo for efficient antigen presentation and CD8 T cell activation. Since the dying infected cell is the origin of a protective T cell response destined to preserve life and individuality, the detour pathway represents an altruistic principle at a cellular level which corresponds to the macroscopic world where death is the precondition to perpetuate the living.

Keywords: apoptosis; CD8 T cells; cross-priming; tuberculosis.

Introduction

Mycobacterium tuberculosis and numerous other intracellular pathogens primarily infect macrophages of the host macroorganism. Since these phagocytes aim at eliminating the infecting agent, pathogens developed evasion mechanisms to escape host defense.[1] Mycobacteria inhibit the maturation of the phagosome at an early endocytic stage and impede subsequent fusion with lysosomes harboring the enzymatic machinery for destruction of pathogens.[2] Central to an efficacious host response in tuberculosis are CD4 T cells. Yet, CD8 T cells also play an important role in protection since they can differentiate into cytotoxic T lymphocytes (CTL) which kill infected target cells and express direct bactericidal activity upon mycobacteria inside cells.[3–5] In addition, activated CD8 T cells secrete the cytokine interferon-γ which induces antimicrobial mechanisms of uninfected macrophages. CD8 T cell activation requires recognition of antigenic peptides presented by MHC-I molecules on antigen presenting cells (APC). MHC-I is loaded in the endoplasmic reticulum (ER) with peptides derived from cytosolic proteins. Since mycobacteria remain enclosed in the phagosome and do not gain access to the cytosol, a critical question has been how CD8 T cells meet their respective mycobacterial antigens. As a solution to this problem, we recently described the detour pathway of T cell activation (Figure 1). According to this concept, mycobacteria induce apoptosis of infected macrophages. During this process of coordinated cell death, macrophages release apoptotic vesicles containing mycobacterial antigens. Subsequently, these extracellular vesicles are taken up by dendritic cells (DCs) which process the antigenic cargo for MHC-I presentation to CD8 T cells.[6,7] This pathway is also valid for the activation of CD1-restricted T cells. CD1 molecules have striking similarities with MHC-I but present lipid antigens instead of peptides.[8] Apoptotic vesicles from *M. tuberculosis* infected macrophages contain several mycobacterial lipids for antigen presentation through CD1 proteins by DCs.

The process which involves failure of direct antigen presentation to T cells by the primary cell producing or harboring the antigen and subsequent transfer of these antigens to a secondary cell able to present is referred to as cross-presentation.[9] T cell activation by cross-presented antigens is known as cross-priming. Broadening this concept, recent data demonstrate that the phagosome of the primary cell is capable of connecting exogenous antigen with the MHC-I pathway by a mechanism of direct cross-presentation.[9] The detour pathway comprehends classical cross-presentation but simultaneously reaches beyond it: Cross-priming by apoptotic vesicles does not merely reflect a route of antigen allocation but a semantically exclusive mechanism for CD8 T cell activation by phagosome-enclosed pathogens.

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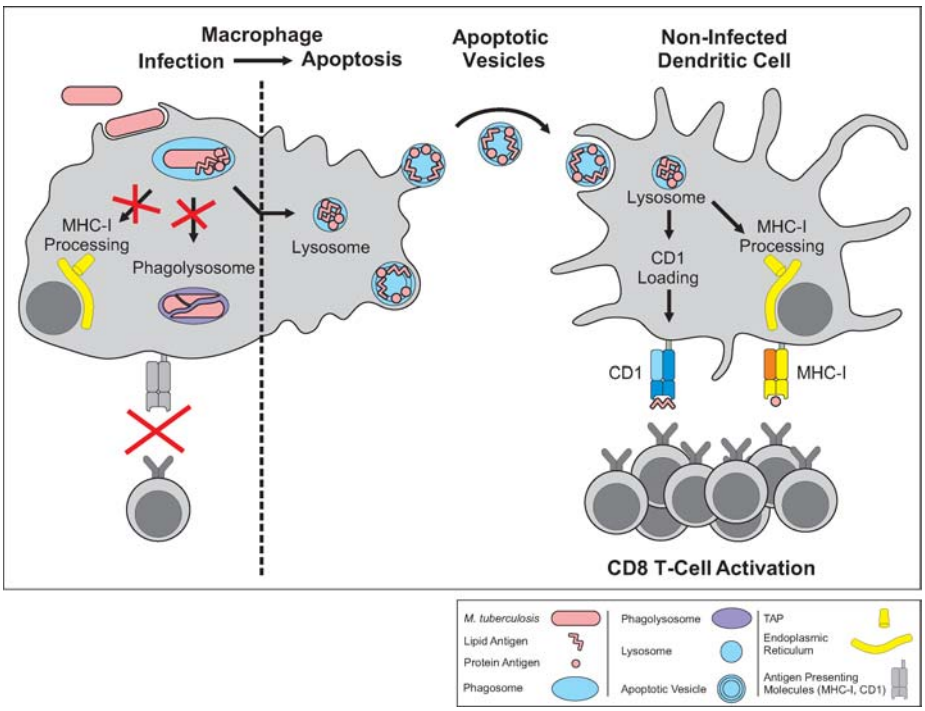
Figure 1. Model of the detour pathway. After infection of macrophages, *M. tuberculosis* inhibits phagolysosome fusion and does not traverse the phagosomal membrane into the cytoplasm. Thus, *M. tuberculosis* remains enclosed in the phagosome and mycobacterial antigens do not gain access to the cytosolic MHC-I pathway for direct presentation to CD8 T cells. However, *M. tuberculosis* induces apoptosis of infected macrophages which subsequently release apoptotic vesicles containing protein and lipid antigens. These blebs are then engulfed by DCs for lysosomal processing. Protein antigens traffic to the cytoplasm by use of a lysosome-to-cytosol transit system unique for DCs to be fed into the MHC-I pathway. Thus, mycobacterial peptides are presented on the surface of DCs for potent MHC-I-restricted CD8 T cell activation. Moreover, CD1 molecules bind mycobacterial lipids in the lysosome of DCs for subsequent CD1-mediated activation of lipid-specific T lymphocytes.



After introducing general aspects of apoptosis, pathogen-induced cell death as well as apoptotic vesicle release, uptake and processing, this treatise concentrates on the different modes of cross-priming.

Abstracting apoptosis

The term apoptosis stems from an ancient Greek word signifying the falling off of something from a larger structure. It was introduced in 1972 by the pathologist John Kerr, who described the orchestrated fashion of cell death which leads to the formation of apoptotic bodies.[10] Thus, apoptosis is primarily a morphological feature of cell death. In contrast to necrosis which is characterized by swelling of cells that eventually leads to rupture and release of cytoplasm inducing inflammation, apoptosis takes place in an ordered manner: The cells shrink, their DNA is degraded, the nuclear as well as the plasma membrane starts to bleb off vesicles and the cytoplasm does not leak into the environment.[11]

Physiologically, apoptosis is vital for tissue homeostasis in maintaining constant cell numbers. During the fade of an antigen-specific T cell response for example, the majority of reactive cells dies by apoptosis limiting the number of circulating T cells to the size of the memory T cell pool.[12] Programmed cell death is not synonymous with apoptosis, although it mostly exhibits an apoptotic phenotype, but represents a term restricted to cell death during development. Robert Horvitz performed his seminal work on apoptosis in development of the round worm *Caenorhabditis elegans* for which he was awarded the Nobel Prize in 2002.[13]

Apoptosis of a cell can be induced through two different routes.[11] The extrinsic pathway reflects the receptor-mediated trigger of cell death. Death receptors (DR) like Fas and TNF-R1 are located in the cell membrane and interact with their binding partners, Fas ligand (FasL) and TNF-α, respectively. The complex formation between Fas and FasL elicits the recruitment of intracellular FADD (Fas-associated death domain protein) which contains death domains (DD) and death effector domains (DED) in order to bind and activate caspases, the crucial proteases of the apoptotic cascade. The multimeric complex of ligand, receptor, adaptor molecule and caspases constitutes the death inducing signaling complex (DISC). Two functional groups of caspases can be distinguished:

initiator caspases like caspase 8 and 10 are proximal of the apoptosis-inducing cascade and function to activate downstream caspases, whereas executioner caspases like caspase 3 and 7 are distal enzymes which cleave cellular substrates mediating the apoptotic phenotype.[14] TNF-R1 exhibits a dual function with respect to signaling; recruitment of FADD to the adaptor molecule TRADD (TNF receptor-associated death domain protein) initiates apoptosis, whereas binding of RIP and TRAF to TRADD induces cellular activation and proliferation. TRAIL (TNF-related apoptosis inducing factor), a ligand for DR 4 and 5, triggers apoptosis upon binding, unless the cell coexpresses decoy receptors for TRAIL naturally present on normal cells and frequently absent from tumour cells.[15]

The second major route of cell death induction is the intrinsic pathway which involves mitochondria as critical functional organelles.[16] Cellular stress such as accumulation of oxidative radicals triggers release of cytochrome C from mitochondria into the cytosol. In complex with Apaf-1 (Apoptotic protease activating factor), caspase 9 and ATP, cytochrome C forms the apoptosome which subsequently activates executioner caspase 3. Cellular substrates for cleavage are ICAD (inhibitor of caspase-activated DNAse) which leads to DNA fragmentation, ROCKI (Rho-associated kinase) resulting in membrane blebbing, PARP (poly-ADP-ribose polymerase) inhibiting DNA repair and actin, which ultimately leads to collapse of the cytoskeleton.[11] Moreover, NF-kB as transcription factor of cellular activation becomes degraded upon caspase action as well as Bcl-2, the most prominent antiapoptotic factor.[17] Effector caspases activate enzymes like sphingomyelinase leading to accumulation of ceramide, a lipid endowed with death-inducing properties.

Inhibitors of apoptosis are the dominant negative form of caspase 8 (FLIP, FLICE-inhibitory protein) which binds to DISC without signaling, Bcl-2 and the IAPs (Inhibitors of apoptosis proteins).[18] Promoters of cell death are the molecules Smac/Diablo which are further factors released from mitochondria and function as inhibitors of IAPs.[16] Mitochondria also segregate AIF (apoptosis-inducing factor) which traffics to the nucleus to elicit DNA fragmentation independently from caspases. Members of the Bcl-2 family like Bad and Bid can have proapoptotic effects as well. They play a role in pore formation in the mitochondrial membrane and subsequent cytochrome C release. Moreover, activated caspase 8 can cleave Bid to generate truncated Bid (tBid) thereby linking the extrinsic with the intrinsic pathway. Hence, the latter pathway may serve as mitochondrial amplification loop for apoptosis.[14]

Pathogen-induced cell death

In response to infection the host develops effector functions which entail apoptosis of cells involved in inflammation. Thus, pathogens indirectly induce cell death of host cells by stimulating the immune system. Activated T lymphocytes, NK cells and activated macrophages secrete inflammatory cytokines like TNF-α which elicit receptor-mediated apoptosis. T cells and NK cells also produce granules containing perforin and granzyme: perforin forms pores in the membrane of target cells paving the way for granzyme to enter the cell for direct activation of the caspase cascade. Moreover, stimulated T cells express FasL which, by binding to Fas, triggers cell death.[19]

In addition, several pathogens including *M. tuberculosis, Salmonella* spp. and *Shigella* spp. directly induce apoptosis.[20] The mycobacterial 19 kDa lipoprotein binds to toll-like receptor 2 (TLR-2),[21] a member of the TLR family of the innate immune system dedicated to sensing the presence of microbes and to initiate host response,[22,23] which results in activation of myeloid differentiation factor 88 (MyD88) followed by recruitment of FADD and caspase 8.[24] In parallel, 19 kDa lipoprotein mediates secretion of the proinflammatory cytokine IL-1ß.[25] *Yersinia* spp. cause cell death of target cells by stimulation of TLR-4 through ligation with bacterial LPS.[26] In contrast, shigella located in the cytoplasm directly activates caspase 1 followed by secretion of inflammatory mediators IL-1ß and IL-18.[27]

Apoptotic vesicle transfer

A hallmark of apoptosis is disintegration of the dying cell and release of vesicles. This also holds true for mycobacteria-infected macrophages which initiate bleb formation upon activation of apoptosis.[6] Cell shrinkage and vesicle budding is realized by permanent contractive interactions between myosin and actin molecules. Caspase-induced cleavage of ROCKI leads to constitutive myosin light chain phosphorylation due to sustained kinase activity of the cleavage product.[28] Not only vesicles are formed during cell death, the membrane texture also changes in a unique way. The apoptotic cascade converges in degradation of flippase, an enzyme critically required for retaining the lipid phosphatidylserine (PS) in the inner leaflet of the cell membrane. This process is accompanied by the activation of scramblase which facilitates surface exposure of PS characteristic for apoptotic membranes.[29]

Apoptotic vesicles in tuberculosis contain a multitude of mycobacterial proteins and lipids, with the 19 kDa lipoprotein and the lipids lipoarabinomannan (LAM) and trehalose dimycolate being of high abundance.[6] These compounds not only confer antigenicity but also adjuvanticity to the vesicles since they are ligands for both T cell receptors (TCRs) and TLRs. After bleb release into the cellular environment, apoptotic vesicles are engulfed by APCs including DCs mainly through interaction between PS on the bleb and PS receptor on the surface of the recipient cell.[30] This notion is supported by the finding that knock-out of PS receptor abrogates clearance of apoptotic

bodies.[31] Additional receptors implicated in uptake of vesicles include the scavenger receptors, ß3 integrins and CD 14.[32,33] Uptake of apoptotic vesicles derived from mycobacteria-infected cells is completely blocked by the addition of RGD peptide from the Tat protein of HIV and soluble CD 14 suggesting a crucial function of ß3 integrins and CD 14 in cross-presentation in tuberculosis.[6] Downstream signaling of PS receptor causes production of immunosuppressive cytokines like TGF-ß and IL-10 which mediate the silencing function of apoptosis.[34] Concerted cell death permanently takes place in every tissue of the body, and hence the macroorganism must avoid responses to apoptotic cells to preclude inflammation and autoimmune disease. Consistent with this notion, necrotic rather than apoptotic cancer cells promote DC maturation for efficient cross-priming of CD8 T cells.[35]

The concept of silent apoptosis however is challenged by infection-induced cell death due to the unique nature of the apoptotic vesicles carrying microbial compounds in addition to host proteins and lipids. These bacterial molecules are highly conserved structures termed pathogen-associated molecular patterns (PAMPs) and include LAM, LPS and bacterial lipoprotein (BLP) which stimulate through TLRs on APCs.[22] Upon TLR ligation, activated APCs mature and express costimulatory molecules as well as inflammatory cytokines for efficient T cell activation. Pathogen-induced apoptotic vesicles transmit a danger signal to the antigen-presenting sentinel thus overriding the silencing component of apoptosis. In short, in infection apoptotic vesicles turn quiet into loud.

Following uptake by DCs, apoptotic vesicles traffic to late endosomes and lysosomes of the recipient cell. In tuberculosis, inhibition of lysosomal acidification by the H^+-ATPase blocker bafilomycin abolishes presentation of apoptotic bleb-derived antigens. Proteasomal processing plays a minor role in antigen presentation of apoptotic vesicles from mycobacteria-infected macrophages.[6] However, recent data analyzing different experimental models of cross-presentation clearly show a fundamental impact of proteasomes and TAP molecules in recipient APCs on cross-priming of CD8 T cells.[36]

Mechanisms of cross-priming

Cross-priming describes the activation of CD8 T cells by recipient APCs which acquired antigen from donor cells. Thus, transfer of material from the cell primarily harboring the source of antigen to the APC is vital for cross-presentation. Extending this view, experiments performed more than a decade ago already suggested that the early phagocytic compartment contains the machinery sufficient for MHC-I-restricted antigen presentation.[37] Phagocytosis of bacteria which failed to penetrate into the cytosol resulted in a vacuolar mechanism of cross-presentation. Thus, in addition to classical cross-priming involving donor and recipient cell, the primary cell containing the antigen is also able to link the phagosome with the MHC-I pathway by a mechanism of direct cross-presentation. However, the precise route antigens take to find their way to the presentation pathway as well as the nature of the antigenic source be it whole proteins, peptides or peptide-chaperone complexes are still matter of a vivid debate.[9]

In 1976, Michael Bevan was the first to characterize the principle of cross-priming in a model demonstrating T cell reactivity to minor histocompatibility antigens.[38] In classical experiments he immunized the F1 generation of mice with different MHC haplotype ($H\text{-}2^b \times H\text{-}2^d$) with spleen cells from either $H\text{-}2^b$ or $H\text{-}2^d$ parental animals. He showed that recipient mice were able to mount an $H\text{-}2^d$-restricted T cell response against antigens from $H\text{-}2^b$ donor cells. Since then, approximately 30 years of research created a comprehensive wealth of knowledge on cross-priming and established a plethora of models.[9]

Recent data indicate that DCs are not only the most powerful APCs but also take a central role in cross-presentation.[39,40] Mainly $CD8^+$ DCs are constitutively programmed for cross-priming *in vivo*.[41] Still, $CD8^-$ DCs can also be induced to cross-present antigen upon stimulation.[42] The presentation capacity is differentially regulated in DCs and depends on the state of maturation.[43] Thus, immature DCs primarily focus on antigen uptake, but even after maturation, DCs need a distinct stimulus for cross-presentation in the form of CD40 ligation which clearly contrasts the mechanisms of direct antigen presentation. In addition to DCs, activated macrophages and B lymphocytes function as cross-presenting cells.[44] Although cross-priming principally focuses on CD8 T cell activation, other T lymphocyte populations including CD1d-restricted NKT cells which recognize lipids as well as CD4 helper T cells can be induced by essentially similar mechanisms.[45] Since the antigen has to be transferred from the donor to the presenting cell, research on vehicles mediating antigen delivery identified heat shock proteins (HSPs) as extracellular carrier molecules and adjuvants.[46,47] Recent work examining knock-out mice deficient in a major HSP transcription factor points at a fundamental role of HSPs in cross-priming.[48]

Though, cross-priming has been convincingly demonstrated in diverse systems using antigen-coated latex beads, tumours and model antigens like ovalbumin, conflicting reports indicate failure of cross-presentation.[49] Rolf Zinkernagel and his group provided evidence in viral and tumour systems against cross-priming as major pathway for CD8 T cell activation. In experiments using tumour cells expressing antigens from lymphocytic choriomeningitis virus (LCMV), CD8 T cells specific

for glycoprotein 33 (GP33) were not induced by cross-priming.[50] Moreover, the majority of *in vivo* studies lack classical F1 experiments especially those with regard to tumour models.[49] The poliovirus apparently fails to directly infect APCs. Yet, viral RNA can be engulfed by APCs for subsequent antigen generation and T cell activation through a process of pseudo cross-priming.[51]

However, novel experiments provide evidence for a consistent and general concept of cross-priming. Vaccination of patients with pancreatic cancer cell lines induced a mesothelin-specific CD8 T cell response.[52] Mesothelin is a tumour-associated antigen expressed my most pancreatic cancers. The antigen-specific CD8 T cells recognized their epitope in the context of HLA molecules which were not present in the cell lines used for vaccination. Moreover, three independent research groups identified the nature of the cross-priming antigen and described it as a mature protein with sufficiently long life-span required for steady-state concentrations in both the donor as well as in the recipient cell of cross-priming. Using fibroblasts transfected with different ovalbumin constructs and subcellular fractionation it was found that the cross-presented antigen is a whole mature protein instead of a peptide or peptides associated with HSPs.[53] Corroborating these findings, it was demonstrated that the cross-presented antigens are proteasomal substrates representing polypeptides. Cross-presenting capacity was markedly increased by treating donor cells with proteasome inhibitors.[54] Finally, the location of an epitope in a nascent protein appears to be decisive for the ability to cross-prime CD8 T cells.[55] Thus, antigens composed of signal peptides fail to stimulate T cells through cross-presentation presumably due to their instability and short life-span. Interestingly, a multitude of CD8 T cell epitopes are derived from signal sequences including the antigenic determinant of GP33 extensively studied in the LCMV system. Taken together, it is not a matter of question whether cross-priming is the rule or the exception, but rather whether a given antigen is qualified to gain access to the pathway of cross-presentation or not. The mechanism of cross-presentation must be robust since antigen quantity required for T cell activation is similar to that needed for classical MHC-II presentation both *in vitro* and *in vivo*.[56]

At least six not mutually exclusive models have emerged to explain the cellular mechanisms of cross-priming which will be described in the following.

1. *Endocytotic exchange*
   MHC-I molecules expressed on the cell surface are internalized by endocytosis and thereby directed to early endosomes. The surface antigen of the hepatitis B virus (HBsAg) is concomitantly endocytosed with empty surface MHC-I proteins[57] and in the early endosome viral peptides are loaded on MHC-I molecules. Moreover, peptides from already loaded MHC-I complexes can be exchanged by endosomal peptides with higher affinity to MHC-I.[58] Recent data indicate that MHC-I molecules contain a tyrosine-based signal motif that mediates trafficking of surface MHC-I to endolysosomal compartments for loading with exogenous peptides.[59] Mutation of this targeting signal abrogates the CD8 T cell response to viral epitopes suggesting a central function of endocytotic exchange in cross-priming of T lymphocytes.
2. *MHC-II-like trafficking*
   At least a subpopulation of MHC-I molecules binds the invariant chain (Ii) which normally associates with nascent MHC-II molecules in the endoplasmic reticulum in order to protect the antigen binding groove from loading with endogenous ligands.[60] Principally, the function of Ii to guide MHC-II to endolysosomal compartments can apply to MHC-I molecules, too. After trafficking of MHC-I/Ii complexes to endolysosomes, MHC-I is prepared for loading with exogenous antigens.
3. *Phagosome egression*
   Few pathogens are capable of escaping from the phagosome after infection or endocytosis. The most prominent example is *Listeria monocytogenes*, a Gram-positive bacterium that induces pores in the phagosomal membrane by means of listeriolysin O (LLO).[61] Thus, *Listeria monocytogenes* gains access to the cytosol and the conventional MHC-I pathway. As a consequence, listerial proteins are available for cytosolic degradation by proteasomes and subsequent processing for MHC-I presentation. Indeed, listeriae are vigorous inducers of CD8 T cell responses with LLO-derived peptides being the immunodominant epitopes.
4. *ER-mediated phagocytosis*
   Based on proteomic analyses of subcellular compartments, it has been shown that the phagosomal membrane contains proteins characteristic for the ER.[62] During phagocytosis of inert particles or pathogens, the ER temporarily fuses with the plasma membrane and thereby assists in the generation of phagosomes. During phagosome maturation, the ER continues to occasionally fuse with the phagosomal membrane providing the endosome with ER-derived molecules. Treatment of cells with bafilomycin which inhibits the vacuolar H^+-ATPase arrests fusions between ER and plasma membrane which then become visible as persistent continuities.[62] This anastomosis promotes direct feeding of exogenous antigens into the ER lumen for further intrinsic processing along the classical MHC-I pathway.
5. *Autonomous phagosome*
   Based on the findings of ER-mediated phagocytosis, recent data demonstrate that the phagosome comprises components originating from the plasma membrane and the ER and constitutes a self-contained organelle

for cross-priming of CD8 T cells.[63,64] The formation of the phagosome is assisted by fusions between ER and plasmalemma which supplies the phagosome with ER-derived proteins. These molecules include all proteins required for MHC-I-mediated presentation like transporters associated with antigen presentation (TAP) and the peptide loading complex consisting of tapasin, calreticulin and Erp57.[65] Moreover, proteasomes are attached to the outside of phagosomes indicating an organelle-associated capacity for protein processing. Thus, after uptake of antigen by APCs, exogenous proteins are assumed to be translocated from the lumen to the surface of the phagosome possibly by Sec61, an ER-derived membrane pore molecule usually involved in retrotranslocation of misfolded proteins to the cytosol.[66] At the outer side of the phagosome, antigenic proteins are degraded by proteasomes to generate peptides which in turn are transferred to the phagosomal lumen by TAP. Thereafter, antigenic peptides can be loaded on MHC-I molecules for subsequent antigen presentation. Taken together, the phagosome represents an autonomous organelle for cross-presentation of exogenous antigens.

6. *Detour pathway*
   Early research on the relationship between apoptosis and CD8 T cell activation by Mathew Albert *et al.* demonstrated that apoptotic cells provide antigen to DCs for cross-priming of CD8 T cells.[67] However, the precise nature of the transfer mechanism remained unspecified. More recent studies described a detour pathway for CD8 T cell activation by antigens derived from phagosome-enclosed pathogens and identified apoptotic vesicles as cross-priming entities.[6,7] Intracellular pathogens like *M. tuberculosis* primarily infect macrophages where they remain confined to the phagosome of the host cell. Because mycobacteria persist enclosed within phagosomes, mycobacterial antigens are secluded from the cytosolic MHC-I pathway. Yet, *M. tuberculosis* induces apoptosis of infected cells which subsequently emit apoptotic vesicles containing mycobacterial antigens. These blebs are then incorporated by DCs which prepare the mycobacterial cargo for presentation to MHC-I-restricted CD8 T cells (Figure 1). The detour pathway also covers the activation of lipid-specific T lymphocytes restricted to CD1 molecules.[68] Apoptosis of infected donor cells was critically required for successful cross-priming of CD8 T cells since inhibition of cell death by a global caspase inhibitor totally abrogated vesicle formation, transfer and subsequent T cell activation.[6] Processing of vesicles in recipient DCs was predominantly associated with lysosomes because treatment of recipient cells with the vacuolar H^+-ATPase inhibitor bafilomycin completely suspended CD8 T cell stimulation. In contrast, blocking of proteasomal processing in APCs had a minor effect on antigen presentation.[6] A crucial step along the detour path in the cross-presenting APC is the passage of antigens from lysosome to cytosol in order to allow MHC-I-mediated presentation. Indeed, DCs in contrast to macrophages are exclusively endowed with a lysosome-to-cytoplasm transit system.[69,70]

Highly different vesicle structures have been thoroughly studied using exosomes from tumour cells and APCs in cross-priming of CD8 T cells.[71] Exosomes represent secreted lysosomes that are continuously released from cells independently from cell death. The detour pathway in tuberculosis does not depend on exosomal transfer of antigens since inhibition of apoptosis in donor cells abrogates cross-priming.[6]

Apoptosis as prerequisite for CD8 T cell activation is also valid in other infection models using intracellular pathogens. Macrophages infected with wild-type *Salmonella typhimurium* inducing apoptosis facilitate cross-presentation by neighbouring DCs in contrast to infection with a mutant strain unable to elicit macrophage cell death.[72] Interestingly, macrophages used as recipient APCs fail to activate T lymphocytes underlining the importance of DCs as the central cell in cross-priming. Although apoptotic vesicles have not been isolated, the detour pathway is most probably operative in Salmonella infection, too. Recent experiments in the *L. monocytogenes* infection model revealed that neutrophils play an important role in cross-priming.[73] Neutrophils are phagocytes and members of a first line of immune defense. *L. monocytogenes* targets neutrophils and induces apoptosis upon infection. Depletion of these cells markedly reduces cross-priming of listeria-specific T cells. Thus, in listeriosis neutrophils could serve as donor cells which release apoptotic vesicles containing listerial antigens for transfer to DCs. Taken together, the detour pathway is probably applicable to a multitude of cross-priming conditions.

Conclusions

The detour pathway comprehends classical cross-priming of CD8 T cells in terms of antigen transfer from a donor cell to the presenting cell. However, the apoptotic vesicle pathway reaches beyond a specific mode of antigen distribution since it allows to circumvent the cul-de-sac of the phagosome trapping intracellular pathogens. Thus, the detour pathway represents a unique instrument for CD8 T cell activation by antigens otherwise segregated in early endocytic compartments.

The relation between apoptosis and CD8 T cell activation in tuberculosis is reflected by the finding that the tuberculosis vaccine strain BCG is a weaker inducer of apoptosis than *M. tuberculosis* which consistently elicits a

Figure 2. Crucifixion. The tableau is by the Master of the Life of Mary around 1470 and located in the Wallraf-Richartz-Museum in Cologne, Germany. Shown is the crucifixion of Jesus of Nazareth at Mount Calvary. Christian religion believes that Jesus Christ is the son of God. On the third day after his burial, he is believed to be resurrected to ascend into heaven for the forgiveness of sins and everlasting life. Next to the cross are Mary (mother of Jesus), John (one of Jesus' disciples) and Mary Magdalene. The cherubs collect the blood in the sense of heritage for life. The photograph appears courtesy of the "Rheinisches Bildarchiv Koeln".

stronger CD8 T cell response. A relation between apoptosis and T cell activation also exists *in vivo* because treatment of established tumours with the apoptosis-inducing chemotherapeutic agent gemcitabine increases the degree of cross-priming.[74] Moreover, experimental tumour therapy using monoclonal antibodies which trigger apoptosis in cancer cells concomitantly amplifies CD8 T cell stimulation.[75] Novel DNA vaccination approaches take advantage of DNA constructs which encode the Fas molecule in addition to the immunogen thereby enhancing apoptosis of the antigen-expressing target cell and subsequent CD8 T cell activation.[76] Taken together, cross-priming mediated by apoptotic vesicles is probably operative *in vivo*. Thus, the detour pathway should be exploited for future vaccination strategies.[77]

Finally, the detour pathway reflects an altruistic principle on a cellular basis since the dying infected cell is the origin of a T cell response aimed at conservation of life and individuality. This is an old principle meaning death is the precondition to perpetuate the living world (Figure 2).

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