Microreview

Apoptosis paves the detour path for CD8 T cell activation against intracellular bacteria

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Summary
Intracellular bacteria such as Mycobacterium tuberculosis primarily infect macrophages. Within these host cells, the pathogens are confined to phagosomes and their antigens are secluded from the classical MHC I presentation pathway. Moreover, macrophages fail to express certain antigen presenting molecules like CD1 proteins. As a result of this intracellular lifestyle, the pathways for the induction of MHC I- and CD1-restricted CD8 T cells by such microorganisms remain elusive. Based on recent findings in tuberculosis and salmonellosis, we propose a new detour pathway for CD8 T cell activation against intracellular bacteria through apoptotic blebs from infected macrophages. Pathogen-derived antigens including proteins and lipids are delivered from infected cells to non-infected professional antigen presenting cells (APC) (Schaible et al., 2003). This detour pathway facilitates cross-presentation of mycobacterial antigens to major histocompatibility complex (MHC) class I- and CD1b-restricted CD8 T cells (Fig. 1). Cross-priming through apoptotic cells has been demonstrated before to be relevant in antiviral and tumour immunity (Albert et al., 1998; Sauter et al., 2000). Because mycobacteria are confined to phagosomes, mycobacterial antigens do not have access to the respective processing/presentation pathways for CD8 T cell stimulation. Thus, the detour pathway is essential to activate CD8 T cells in tuberculosis. This concept can be extended to infections with other intracellular bacteria such as salmonella (Yrlid and Wick, 2000; Yrlid et al., 2001). As cell death is widely regarded as detrimental for the host, we consider apoptosis as a result of infection with intracellular bacteria a beneficial gateway to promote protective immunity.

(1) Apoptosis: a basic physiological process goes micbicidal
In this review, we propose a new function of apoptosis in antibacterial immunity. Intracellular bacteria such as mycobacteria and salmonellae induce apoptosis in their host macrophages (Navarre and Zychlinsky, 2000; Yrlid and Wick, 2000; Menaker and Jones, 2003). We recently characterized a novel detour pathway of how antigens from phagosome-enclosed mycobacteria are shuttled by the means of apoptotic blebs from infected macrophages to non-infected professional antigen presenting cells (APC) (Schaible et al., 2003). This detour pathway facilitates cross-presentation of mycobacterial antigens to major histocompatibility complex (MHC) class I- and CD1b-restricted CD8 T cells (Fig. 1). Cross-priming through apoptotic cells has been demonstrated before to be relevant in antiviral and tumour immunity (Albert et al., 1998; Sauter et al., 2000). Because mycobacteria are confined to phagosomes, mycobacterial antigens do not have access to the respective processing/presentation pathways for CD8 T cell stimulation. Thus, the detour pathway is essential to activate CD8 T cells in tuberculosis. This concept can be extended to infections with other intracellular bacteria such as salmonella (Yrlid and Wick, 2000; Yrlid et al., 2001). As cell death is widely regarded as detrimental for the host, we consider apoptosis as a result of infection with intracellular bacteria a beneficial gateway to promote protective immunity.

(2) Programmed cell death and signalling
Apoptosis, or programmed cell death, is essential for removal of cells from tissues, after they have fulfilled their functions. Thus, apoptosis is a basic physiological function during embryonic development, growth and tissue homeostasis to sustain the integrity of a macroorganism and its organ systems. In the immune system, self-reactive lymphocytes are deleted by apoptosis and, in the course of an ongoing immune response, apoptosis is involved in limiting numbers of activated lymphocytes before reaching immunopathological quantities with detrimental outcome for the host. Furthermore, potent defence cells with potentially destructive effector mechanisms, notably neutrophilic granulocytes, are restrained by apoptosis to a naturally short life span.

Signals, which drive cells into apoptosis, are mediated by surface receptors, so-called death receptors (DR)
(reviewed in Bhardwaj and Aggarwal, 2003). These belong to the tumor necrosis factor receptor superfam-
ily (TNFR) including TNFR1 (DR1) and 2, Fas (DR2), DR3–6 and CD40, and share a stretch of about 80 amino acids termed death domain (DD). This domain is required for apoptotic signal transduction and is also present in intra-
cellular proteins involved in apoptosis induction and signalling. Apoptosis is elicited by a number of physiological stimuli, including TNF, Fas ligand (FasL) and TRAIL, which bind to TNFR1, Fas, or DR4/5 respectively. Specificity of these stimuli depends on the cellular distribution of the receptors. Ligand-binding to DR elicits two independent intracellular signalling cascades: one is represented by FasL/Fas interaction involving RIP and release of cyto-
chrome c from mitochondria and leading to Pro-caspase 9 activation; the other one is exemplified by the TNF/
TNFR1 pathway with participation of TRADD, TRAF2 and FLICE directly targeting caspase 3/7 (Bhardwaj and
Aggarwal, 2003). The subsequent caspase cascade leads to degradation of proteins involved in the maintenance of cellular functions and inhibition of apoptosis. Thereby cell shrinking, chromatin condensation, DNA fragmentation, blebbing of nuclear and plasma membrane and finally cell death is triggered. Activated caspase 9, as well as caspase 6, cleave and activate caspase 3, which in turn cleaves various substrates including the poly/ADP-ribose polymerase (PARP), NF-κB, protein kinase C (PKC), the apoptosis inhibitor Bcl-2 and the caspase-activated DNAse ICAD. Activation of ICAD is finally responsible for apoptosis-associated DNA degradation. Caspase 3 also activates scramblase activity through the cleavage of PKC-δ and inactivation of the aminophospholipid translo-
case thus allowing permanent phosphatidyl serine (PS) exposure on apoptotic bodies (Bhardwaj and Aggarwal, 2003). Phosphatidyl serine is commonly used as apop-
totic cell marker by its affinity to annexin V. A hallmark of apoptosis is the formation of apoptotic blebs or vesicles (Wilhelm and Hacker, 1999). This morphological feature reflecting cellular disintegration critically depends on the rearrangement of cytoskeletal elements: the cells contract through myosin II/actin interaction upon myosin light chain phosphorylation by myosin light chain kinase and the small GTPase Rho (Mills et al., 1998). Furthermore, early during apoptosis, intermediate filaments are formed by caspase 3-dependent interactions between DD-contain-
ing DNA-binding protein (DEDD) 1 and DEDD 2, but finally collapse during cellular disintegration (Lee et al., 2002).

During infection, apoptosis can be caused either by host factors such as proinflammatory cytokines or either Fas/FasL or perforin/granzyme-mediated killing of infected cells by cytotoxic T cells and NK cells, or by the microor-
ganism itself (Navarre and Zychlinski, 2000; Grassme et al., 2001; Menaker and Jones, 2003). A number of intracellular bacteria including M. tuberculosis, M. avium, Salmonella sp. and Shigella sp. induce apoptosis within their host macrophages (Rojas et al., 1997; Klingler et al., 1997; Navarre and Zychlinski, 2000; Yrlid and Wick, 2000; Yrlid et al., 2001; Schaible et al., 2003). The in vitro find-
ings on mycobacteria have been corroborated in vivo by demonstrating programmed cell death in tuberculous granulomas (Fayyazi et al., 2000). Several microbial products have been identified to trig-
ger apoptosis of host cells. Bacterial lipoproteins such as the mycobacterial 19 kDa lipoprotein bind to the toll-like-
receptor 2 (TLR2) and signal through myeloid differentiation factor 88 (MyD88) (Lopez et al., 2003). Thereby, the caspase 8/Fas-associated DD protein (FADD) pathway to cell death is activated. Similarly, Gram-negative bacteria such as Yersinia sp. induce apoptosis in macrophages by signalling through TLR4 probably via lipopolysaccharide (LPS) (Haase et al., 2003). This makes the TLRs true ‘Death Receptors’.

(3) Apoptotic blebs and antigenic cargo

As an important morphological feature during apoptosis, cells disintegrate into blebs. Also mycobacteria-infected APC release extracellular vesicles during their progression towards apoptotic cell death (Schaible et al., 2003). Importantly, these vesicles enwrap mycobacterial proteins as well as cell wall lipids. The mycobacterial 19 kDa lipoprotein represents a predominant protein within apoptotic blebs. This protein trafficks from the phagosome to other compartments within the infected cell, activates macrophages through TLR2 and contains CD8 T cell epitopes (Herrmann et al., 1996; Lopez et al., 2003). Apoptotic blebs from mycobacteria-infected macrophages contain known CD1b lipid ligands such as lipoarabinomannan (LAM) and trehalose dimycylate (Schaible et al., 2003). These mycobacterial cell wall lipids are shed from the bacteria into the phagosomal lumen and are further transferred to late endosomes/lysosomes of the infected cell (Beatty et al., 2000; Schaible et al., 2000; Fischer et al., 2001). Of note, the apoptotic blebs from mycobacteria-infected cells contain multilamellar bodies characteristic for intralysosomal membrane stacks (Schaible et al., 2003).

(4) Phagocyte receptors and apoptosis: a matter of silence

Following the onset of programmed cell death, apoptotic bodies and blebs are engulfed and digested by neighbouring cells. Both, macrophages and DC play a prominent role in this process and both cell types are also deeply involved in activation of antigen-specific T cells. A number of receptors have been identified on the phagocyte surface, which bind apoptotic membrane structures including PS (reviewed in Henson et al., 2001). The PS receptor (PSR) seems to be the most important receptor because blocking PS exposure on apoptotic bodies strongly prevents their uptake. Other receptors involved in uptake of apoptotic bodies/blebs include the broad scavenger receptor family, β1, β2 and β3 integrins and CD14 (Zocchi et al., 1997; Devitt et al., 1998; Ronchetti et al., 1999; Henson et al., 2001). Soluble molecules such as collectins (surfactant proteins, conglutinin, mannose binding protein) can also bind apoptotic blebs. Coated with these molecules, blebs are caught by phagocytes through a surface calreticulin in concert with CD91 (α, macroglobulin receptor), the latter one providing the transmembrane domain for this molecular ‘ménage a trois’ (reviewed in Henson et al., 2001). Uptake of apoptotic blebs derived from mycobacteria-infected macrophages can be inhibited by RGD peptide or soluble CD14 suggesting involvement of β3 integrins such as the vitronecrosis receptor and CD14 in cross-presentation of mycobacterial antigens (Zocchi et al., 1997; Devitt et al., 1998; Schaible et al., 2003).

Phagocyte receptors do not only bind apoptotic bodies but can also transmit silencing signals to the phagocyte. Signalling through the PSR engaged by PS leads to the secretion of transforming growth factor (TGF) β, interleukin 10 (IL-10) and prostaglandin E2 (PGE2) and subsequent suppression of inflammatory mediators (Henson et al., 2001). Uptake of apoptotic bodies does not trigger maturation of DC and their migration to the lymphnodes. Therefore, these silencing mediators render apoptotic bodies non-inflammatory to avoid immune recognition and potential autoreactivity. In contrast to apoptosis, necrosis – the other way, how cells can die – does not lead to the formation of blebs but rather results in cellular debris, which causes inflammation and mediates a ‘danger’ signal. In tumour models, it has been shown that only necrotic tumour cells but not apoptotic ones stimulate DC maturation leading to optimized cross priming of CD8 T cells (Sauter et al., 2000). During necrosis, cytoplasmic annexin V and PS-degrading enzymes have been implicated in inhibition of PS-mediated silencing mechanisms, which are active in apoptotic blebs (Henson et al., 2001).

However, a different picture arises in infection-induced apoptosis because microbial pathogen associated molecular patterns (PAMP) contained within apoptotic material can provide inflammatory stimuli by binding to TLR (Takeda et al., 2003). Thereby, T cell polarizing cytokines such as IL-12, IL-18 and chemokines, as well as DC maturation and migration to the draining lymphnodes are induced (Mazzoni and Segal, 2004). For example, apoptotic blebs from mycobacteria-infected macrophages contain an array of cell wall glycolipids and lipoproteins. Amongst these, LAM and the 19 kDa lipoprotein are ligands for TLR2 (Lopez et al., 2003), and blebs from salmonella-infected cells probably carry LPS, a ligand for TLR4 (Takeda et al., 2003). Furthermore, salmonellae and shigellae directly induce macrophage apoptosis through caspase 1 activation (Navarre and Zychlinski, 2000). This protease is also critical for the generation of the proinflammatory cytokines IL-1β and IL-18, which could help to overcome silencing accompanied by uptake of apoptotic bodies. Microbial PAMP may render otherwise immunologically silent apoptotic blebs immunogenic to send potent APC to the draining lymphnode for T cell priming. In summary, infection-induced apoptosis appears

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(5) Intracellular microbes and antigen presenting pathways

In infections with intracellular microbes, the acquired immune response is dominated by T cells which recognize antigens in the context of the antigen presenting molecules MHC I, MHC II, CD1a, CD1b, CD1c and CD1d (Schaible et al., 1999; Kaufmann, 2001). MHC I-restricted T cells are CD8 positive and MHC II-restricted ones carry CD4. The T cells controlled by the different CD1 molecules express either CD4, CD8, both or none of these co-receptors. The MHC I- and MHC II molecules present peptides derived from proteolytic processes within either the cytoplasm or lysosomes respectively. In contrast, CD1 molecules bind lipids for presentation to T cells. The group I CD1 molecules, CD1a, CD1b and CD1c, can present lipid antigens originating from the cell wall of mycobacteria but also self-lipids (Porcelli and Modlin, 1999; Vincent et al., 2003).

For MHC I presentation, proteasomes in the cytoplasm generate antigenic peptides, which are subsequently transported into the endoplasmic reticulum (ER) for binding to MHC I molecules. MHC II molecules bind antigenic peptides from exogenous proteins within late endosomes/lysosomes where they are processed by lysosomal enzymes such as cathepsins or asparaginyl endopeptidase (Schaible et al., 1999). Evidently, microbes residing in phagosomes predominantly activate MHC II-restricted CD4 T cells, whereas MHC I-restricted CD8 T cells are primarily induced by the phagosome escapees L. monocytogenes, Rickettsia sp. or T. cruzi. However, phagosome-confined microbes such as mycobacteria, salmonellae, brucellae, chlamydiae, leishmaniae and plasmodia also induce CD8 T cells, which participate in protection against infections with these pathogens (reviewed in Schaible et al., 1999). The CD8 T cells do not only produce interferon γ (IFNγ) to activate macrophages. They also lyse infected cells via perforin, and simultaneous release of granulysin directly kills intracellular bacteria and parasites (Stenger et al., 1998a; Cho et al., 2000).

Modes of cross-priming

Although it is obvious how cytoplasm-resident microbes feed their antigens into the cytoplasmic MHC class I processing pathway, the route to MHC I molecules for antigens from microbes segregated in phagosomes is difficult to envisage (Canaday et al., 1999; den Haan and Bevan, 2001). Mycobacteria reside in early endosomal compartments (Russell, 2001), and experiments employing co-loading of tracers or radioactively labelled bacteria revealed only minute amounts of material in the cytoplasm (Schaible et al., 2003).

Several pathways have been proposed to explain the enigma of how intraphagosomal microbes can induce MHC I-restricted CD8 T cells (reviewed in Schaible et al., 1999; Rock, 2003):

(i) MHC I molecules can acquire peptides from exogenous antigens outside of the ER. Peptides can be exchanged because of higher affinity compared to endogenous ones during MHC I trafficking through the endosomal system, or on the plasma membrane upon peptide regurgitation (Jondal et al., 1996). Some surface MHC I molecules are devoid of peptides, and others associate with the invariant chain, which targets MHC II molecules into the endosomal system (Sugita and Brenner, 1995; Schirmbeck and Reimann, 1996). Microbial peptides may be caught by such MHC I molecules for presentation.

(ii) Bacterial factors similar to listeriolysin, which permits listeria’s escape from the phagosome to the cytosol (Mazzaccaro et al., 1996; Beauregard et al., 1997), or the type III secretion apparatus of various Gram-negative bacteria, may allow membrane traversing. This ‘injection needle-like’ structure can be inserted into the phagosome membrane and ‘inject’ bacterial effector proteins into the cytoplasm.

(iii) Because of the proposed ER-origin of phagosomal membranes, the phagosome is already equipped with the full machinery for MHC I-processing and -peptide loading including proteasomes, TAP (transporter associated with antigen presentation) molecules and MHC I. It has been suggested that phagosomal antigens can directly be acquired by adjacent MHC I molecules for transport to and presentation on the cell surface. Thereby the classical MHC I pathway would be directly hooked to phagocytosis (Ackerman et al., 2003; Desjardin, 2003; Guermonprez et al., 2003; Houde et al., 2003). However, this model has to be demonstrated for bacterial phagosomes, and still requires a phagosome-to-cytosol pathway (Rodriguez et al., 1999).

(iv) Infection-induced apoptosis causes release of apoptotic blebs, which subsequently transport antigens to non-infected professional APC (Schaible et al., 2003). There, apoptotic material is processed within the endosomal system and may even leak into the cytosol (Bellone et al., 1997). In this scenario, the DC is of critical importance because of its lysosome-to-cytosol transit system, which directs antigens into the classical MHC I presentation pathway (Rodriguez et al., 1999).

These four mechanisms are not mutually exclusive but rather contribute all to MHC I presentation. Yet, based on
Bacteria-induced apoptosis and T cell activation

Recent studies in tuberculosis and salmonellosis we consider the apoptotic bleb-mediated detour pathway the most likely scenario for CD8 T cell activation to antigens from microbes secluded within phagosomes (Yrlid and Wick, 2000; Yrlid et al., 2001; Schaible et al., 2003). The detour pathway further comprehends classical cross-presentation of microbial antigens through MHC I, which represents the basis of effective immune responses despite loss of antigen presenting capacity of primary infected cells. This is of importance as interference of intracellular bacteria and parasites with antigen presentation has been demonstrated for a number of infections (reviewed in Schaible et al., 1999). Moreover, cross-priming provides a model for induction of MHC II- or CD1-restricted T cells despite the absence of the respective antigen presenting molecules on infected cells. MHC II is exclusively expressed on professional APC such as activated macrophages, B cell blasts or DC, and CD1 molecules are primarily present on infected cells. Hence, cross-presentation constitutes a prerequisite for T cell priming against antigens from pathogens, which dwell inside non-professional APC, i.e. Chlamydia sp., Bartonella sp., Ehrlichia sp., Rickettsia sp. and T. gondii.

**Processing of apoptotic blebs**

Upon uptake by DC, extracellular vesicles from mycobacteria-infected macrophages are delivered to late endosomal/lysosomal compartments of non-infected DC (Fig. 2). Integrity of these compartments is prerequisite for presentation of antigenic cargo from apoptotic blebs of mycobacteria-infected macrophages because inhibition of acidification by the vesicular H⁺-ATPase blocker bafilomycin abolished antigen presentation (Schaible et al., 2003). In contrast, proteasomal processing appears dispensable for presentation of mycobacterial antigens within apoptotic blebs. Although antigen processing prior to bleb formation cannot be excluded, lysosomal processing within the non-infected APC seems to be of major importance for cross-presentation. Apart from direct processing of the antigen, lysosomal enzymes may also liberate the antigenic cargo from the blebs membranes. Similarly, salmonella antigens in apoptotic bleb fail to bind to fixed DC for direct presentation and therefore require further processing within the DC (Yrlid and Wick, 2000; Yrlid et al., 2001). This is in contrast to a recent report on the MHC I cross-presentation pathway for cell-associated viral proteins, which depends on both lysosomal cathepsin D activity as well as functional proteasomes and TAP (Fonteneau et al., 2003).

(6) **Dendritic cell is the mother of cross-priming**

Dendritic cells are the prime APC responsible for cross-presentation of antigens associated with apoptotic blebs from infected macrophages in tuberculosis and salmonellosis (Yrlid et al., 2001; Schaible et al., 2003). Upon antigen contact and additional stimulation through TLR, tissue resident DC mature and migrate to the draining lymph-nodes for T cell stimulation. Mature DC express high densities of MHC I, MHC II, all CD1 molecules as well as the co-stimulatory molecules CD40, CD54, CD80 and CD86 (Shortman and Liu, 2002). In salmonellosis, DC seem to be crucially involved in cross-priming to T cells whereas non-infected macrophages compete for apoptotic blebs without contributing to T cell activation (Yrlid et al., 2001).

**Fig. 2.** Immunofluorescence confocal laser micrographs showing labelled extracellular vesicles from *M. tuberculosis* infected macrophages engulfed by human dendritic cells and delivered to late endosomal/lysosomal compartments. The green label is the late endosomal/lysosomal marker LAMP-1 as detected by a specific antibody; the red label for the apoptotic cell membrane is PKH26. Vesicles were incubated with DC for 30 min in the cold, washed and further incubated for another 60 min at 37°C. The yellow colour indicates co-localization of LAMP-1 and apoptotic blebs in late endosomes/lysosomes (pictures from two different experiments with a similar experimental set up are shown).
This could be explained by the lack of co-stimulatory molecules on resting macrophages and/or the lysosome-to-cytosol antigen transfer system exclusively present in DC. A recent study revealed that direct MHC I presentation and cross-presentation are differently regulated (Delamare et al., 2003). Whereas MHC I presentation of endogenous antigens is already performed by immature DC, cross-presentation of an exogenous soluble antigen, i.e. ovalbumin, depends on DC activation by CD 40 ligation. In the context of bacterial infections, presentation of bacterial antigens within apoptotic blebs can rely on microbial compounds such as LPS, LAM or lipoteichoic acids, which signal through TLRs and other pathogen recognition receptor (PRR) thus activating DC (Mazzoni and Segal, 2003). Based on surface marker phenotype, tissue distribution, cytokine production and the capacity to stimulate T cells, DC subpopulations can be distinguished. These subpopulations comprise the myeloid, the lymphoid, the T cells, DC subpopulations can be distinguished. These subpopulations comprise the myeloid, the lymphoid, the plasmacytoid DC and the Langerhans cells of the epidermis (Shortman and Liu, 2002). In mice, differences in the capability to cross-present exogenous antigens have been demonstrated with lymphoid CD8+ DC being superior to myeloid CD8+ DC (den Haan et al., 2000). Taken together, DC represent the predominant professional APC involved in cross-presentation of antigens from intracellular bacteria through the apoptotic bleb detour pathway.

(7) Detour pathway in disease

Apoptotic bodies and blebs thereof serve as important sources of antigens for MHC I-mediated cross-presentation in antiviral and tumour immunity (Albert et al., 1998; Ronchetti et al., 1999; Bellone, 2000; Sauter et al., 2000; Larsson et al., 2001). In addition to apoptotic blebs, small extracellular vesicles probably derived from excreted lysosomes and termed exosomes, have been implicated in antigen transfer for cross-presentation and have successfully been used in antitumour vaccination (Denzer et al., 2000; Therèy et al., 2002). Exosomes from mycobacteria-infected macrophages contain mycobacterial antigens and therefore can participate in antigen delivery to non-infected DC (Beatty et al., 2000). However, we consider apoptotic blebs central to mycobacteria-specific stimulation of T cells because inhibition of apoptosis in infected macrophages by a global caspase inhibitor significantly reduces transfer of antigens to APC and subsequent activation of CD8 T cells (Schaible et al., 2003). Addition of non-infected DC to infected macrophage cultures was indispensable for potent CD8 T cell stimulation through MHC I as well as CD1b. Of note, preliminary in vivo data in mice revealed that blebs from mycobacteria-infected macrophages are efficiently engulfed by DC upon subcutaneous application and are carried to the draining lymphnodes, where antigen-specific CD8 T cells are activated (F. Winau, S. Weber, S. Sad, S. H. E. Kaufmann and U. E. Schaible, unpublished results).

We regard the detour pathway for CD8 T cell activation in tuberculosis biologically significant as mycobacteria are confined to early endosomal phagosomes and therefore mycobacterial antigens have no direct access to the respective processing/presentation pathways for CD8 T cell stimulation (Schaible et al., 2003). Thus, cross-priming by apoptotic blebs is not only a way of antigen delivery, but an essential immunological function due to the biology of mycobacteria. Hence, it is challenging to assume that the apoptosis-mediated detour pathway for antigen presentation represents the conditio sine qua non for CD8 T cell stimulation during infections with bacteria secluded within phagosomes. Furthermore, we consider cross-priming to be important in tuberculosis because mycobacteria-infected APC rapidly loose their antigen presenting capacity upon infection. Moreover, macrophages, the primary host cells for mycobacteria, a priori do not express CD1 molecules. These lipid-binding molecules, however, are abundant on DC. Although mycobacteria can infect DC (Jiao et al., 2002), CD1b expression and induction of CD1b-mediated T-cell response by DC are inhibited as well upon infection (Stenger et al., 1998b). In conclusion, non-infected DC are the key APC for mycobacteria-specific T cell responses.

Apoptosis has also been demonstrated to be critical for the induction of CD8 T cells in murine salmonellosis. Only a S. typhimurium strain, which induces programmed cell death, stimulates CD8 T cells (Yrid et al., 2001). Interestingly, the salmonella strain, which induces apoptosis, also expresses a functional typ III secretion system. Because bystander DC are critical for cross-presentation of salmonella antigens through MHC I, an apoptotic bleb mediated process can be assumed as underlying mechanism rather than direct antigen delivery into the cytoplasm by salmonella’s own ‘injection needle’ (see above).

In this context, it is notable that M. tuberculosis is superior to the antituberculosis vaccine strain M. bovis BCG in eliciting apoptosis. Consistent with this finding, BCG is a weak inducer of CD8 T cells, which has been taken as one explanation for the low efficiency of BCG vaccination (Kaufmann, 2001). The findings in salmonellosis and tuberculosis provide compelling evidence that cross-priming through apoptotic blebs is a critical prerequisite for CD8 T cell-dependent host defense. Future studies have to determine the general relevance of this detour pathway for the activation of CD8 T cells in infections with other bacteria and parasites.

(8) ‘Trojan horse’ unsaddled

A number of intracellular bacteria trigger apoptosis in host
cells. However, some intracellular microbes such as Chlamydia sp. and Leishmania sp. delay naturally occurring apoptosis in neutrophils, the first effector cells they encounter. It has been proposed that microbes abuse apoptotic bodies as ‘trojan horses’ to enter their final destination, the macrophage (Laskay et al., 2003). Inhibition of apoptosis by these microbes is still incompletely understood apart from the induction of antiapoptotic molecules, notably bcl2. It has been speculated that, enveloped in apoptotic bodies, pathogens enter macrophages undetected by PRR as the result of the immunologically inert composition of apoptotic bodies. As discussed here, pathogens contain PAMP such as glycolipids and/or bacterial CpG-rich DNA, which can activate macrophages through PRR. The ‘trojan horse’ hypothesis therefore depends on the pathogen’s ability to minimize the amount of PAMP to avoid induction of microbicidal mechanisms. We provide a different view of how delaying host cell apoptosis can favour the pathogen’s survival. By diminishing spread of antigens through apoptotic blebs to non-infected professional APC, T cell activation will be limited. In concert with down-modulation of antigen presentation and inhibition of antimicrobial effector functions in the infected host cell itself, this mechanism may leave the intracellular microbe unrecognized by the immune system to establish infection or to progress to latency rather than being eliminated. In these cases, delay of apoptosis can be viewed as a virulence-associated strategy to restrict cross-presentation.

(9) Detour path to direct therapy

Together with AIDS, tuberculosis and malaria are the most deadly human infections, and infectious diseases caused by other intracellular microbes such as typhoid fever, trachoma and leishmaniasis further increase the burden worldwide causing major public health problems. Therefore, elucidation of the detour path for antigen presentation through MHC-I and CD1 can provide guidance for rational design of novel vaccination strategies against intracellular pathogens (Restifo, 2000). To enhance apoptosis in infected cells, vaccine strains such as BCG or S. typhimurium Ty2 or DNA vaccines could be equipped with apoptosis-inducing properties to target professional APC. This strategy will not only promote priming of MHC-I and CD1-restricted CD8 T-cells, but also potentiates stimulation of MHC II-restricted CD4 T cells. However, the microbial factors responsible for the elicitation of apoptosis remain ill-defined. For DNA vaccines, a delivery system has been constructed, which allows co-expression of antigens and Fas in vivo (Chattergoon et al., 2000). Indeed, this construct enhanced antigen-specific CD8 T cell responses. Co-administration of antibodies to Fas together with an antitumour vaccine has been found to increase the vaccine efficiency probably based on similar mechanisms (Ludwig et al., 2003). In conclusion, apoptosis induced by intracellular microbes represents an important step towards efficient T cell induction by cross-presentation. This detour pathway should be applied to future vaccination strategies against such threatening infections as tuberculosis, typhoid fever and malaria.

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