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Review Article

Macrophage Apoptosis in Tuberculosis

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Mycobacterium tuberculosis (*Mtb*) is an intracellular pathogen that infects alveolar macrophages following aerosol transmission. Lung macrophages provide a critical intracellular niche that is required for *Mtb* to establish infection in the human host. This parasitic relationship is made possible by the capacity of *Mtb* to block phagosome maturation following entry into the host macrophage, creating an environment that supports bacillary replication. Apoptosis is increasingly understood to play a role in host defense against intracellular pathogens including viruses, fungi, protozoa and bacteria. In the last 15 years an understanding of the role that macrophage apoptosis plays in TB has begun to emerge. Here we review the history and current state of the art of this topic and we offer a model of the macrophage-pathogen interaction that takes into the account the complexities of programmed cell death and the relationship between various death signaling pathways and host defense in TB.

Key Words: Tuberculosis, macrophage, apoptosis, mycobacterium, host defense

INTRACELLULAR PARASITISM BY TUBERCLE BACILLI

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), is a facultative intracellular parasite of macrophages. The bacillus is non-motile and lacks the secreted toxins used by extracellular bacterial pathogens to fashion an environment suitable for growth in the infected host. In order for *Mtb* to establish infection it must first gain entry into resident alveolar macrophages following inhalation of infectious aerosols. Macrophages patrolling the distal airways avidly engulf inhaled bacteria using a variety of phagocytic receptors. A number of different phagocytic receptors have been implicated in *Mtb* entry to macrophages, with complement receptor and mannose receptor likely the predominant pathways.[1,2] After phagocytosis, non-pathogenic bacteria are degraded by the acidification of the phagosomal compartment and its subsequent fusion with lysosomes that contain hydrolases active at low pH. Keys to the virulence of *Mtb* are its capacity to prevent the incorporation of the ATP/proton pump into the phagosome membrane and to restrict the fusion of this vacuole with lysosomes.[3] Protected in a compartment with features of an early endosome, tubercle bacilli are capable of replication unless their growth is restricted by interferon (IFN)-γ mediated activation of the host macrophage.[4]

An initial phase of intracellular growth in lung macrophages is required for *Mtb* to establish productive infection in the host. This point was demonstrated in experiments where resident alveolar macrophages were depleted in mice using liposome-encapsulated dichloromethylene diphosphonate prior to aerosol infection with *Mtb*.[5] Mice depleted of alveolar macrophages were relatively protected from *Mtb* infection, whereas the same macrophage depletion method dramatically increased the susceptibility of mice to infection with *Streptococcus pneumoniae*.[6] These findings indicate that alveolar macrophages play a protective role in host defense against a typical extracellular bacterial pathogen but facilitate infection with *Mtb* at least until they can be activated by IFN-γ provided from T cells.

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In later stages of active pulmonary TB the bacilli can adopt an extracellular lifestyle in foci of necrosis. Lung cavities that connect to airways provide an oxygen-rich environment permitting extracellular *Mtb* to reach high densities and an open pathway for transmission.[7] Lacking an environmental reservoir *Mtb* depends on aerosol transmission between human hosts for its persistence, highlighting the importance of the transition from intracellular to extracellular infection. From these considerations it is clear that the interaction between *Mtb* bacilli and host macrophages is a central element of TB pathogenesis.

APOPTOSIS AS A DEFENSE AGAINST INTRACELLULAR PARASITISM

When confronted with a pathogen that uses host cellular resources for survival and replication one strategy for defense is to activate the programmed death (apoptosis) of the host cell. Apoptosis in response to intracellular parasitism by viruses is a well established paradigm in biology.[8] Many successful viral pathogens encode genes whose products suppress apoptosis of the host cell, thereby sustaining the niche for viral replication.[9-13] The extension of this paradigm to intracellular bacterial pathogens is more recent but a large number of cases have now been identified, including macrophage infection by *Mtb* as discussed below.

Apoptosis is a highly regulated process of cellular deconstruction that confines the cytoplasmic contents of dying cells within membrane bound vesicles (apoptotic bodies) that express "eat me" signals on their surface. Apoptotic bodies are recognized and avidly engulfed by professional phagocytes via a number of specific cell surface receptors; a process called efferocytosis. Binding of apoptotic bodies typically stimulates the expression of anti-inflammatory cytokines including transforming growth factor-β and interleukin-10 (IL-10).[14,15] By suppressing inflammation these cytokines are thought to help limit the tissue damage that might occur if intracellular contents, particularly degradative enzymes, were released to the extracellular space. Apoptosis of infected cells might benefit the host in several ways. It eliminates a protected intracellular environment favorable for replication, forcing the infecting pathogen to reestablish residence in a naïve host cell. In addition to orchestrating the quiet elimination of parasitized cells, packaging of pathogen-specific molecules in apoptotic bodies serves as an efficient pathway for the delivery of antigens following efferocytosis by immature dendritic cells.[16] It is also recognized that in some circumstances infection-induced apoptosis might serve the interests of the pathogen rather than the host. Potential mechanisms for apoptosis associated with disease promotion include the elimination of vital host defense cells, penetration of epithelial barriers and dissemination of infection by the delivery of pathogens to naïve host phagocytes engulfing apoptotic corpses.[13]

CELL DEATH PATHWAYS

Before discussing apoptosis of *Mtb*-infected macrophages it may be helpful to briefly review the major pathways and modes of programmed cell death. Caspases belong to a family of cysteine proteases whose functions are central to the initiation and execution of many forms of apoptosis. Members of the caspase family share structural characteristics (they are expressed as proenzymes) and substrate specificities, cleaving targets with similar tetrapeptide motifs having aspartic acid in the P1 position.[17] Procaspases are activated by proteolytic processing at an internal aspartic acid residue, which may result from autoproteolysis, cleavage by different caspases or cleavage by non-caspase proteases. Caspase activation and subsequent apoptosis can be triggered by at least three distinct pathways. The extrinsic apoptosis pathway is induced after ligation and oligomerization of tumor necrosis receptor (TNFR) family cell surface receptors including TNFR1 and Fas (among others) by their cognate ligands tumor necrosis factor (TNF)-α and Fas ligand (FasL).[18] A death-inducing signal complex (DISC) is then formed by recruitment of Fas-associated death domain and the initiator procaspases-8 or -10 that are autoactivated at the DISC. These initiator caspases trigger a cascade of downstream caspases through proteolytic processing of their precursor

zymogens, terminating with the activation of effector caspases including caspase-3, -6 and -7 whose functions, along with a number of non-caspase enzymes, result in nuclear condensation, DNA cleavage and the formation of apoptotic vesicles.[19]

The intrinsic apoptosis pathway is induced by intracellular stresses such as DNA damage, nutrient deprivation and oxidative stress. These triggers promote mitochondrial outer membrane permeability, permitting cytosolic translocation of cytochrome c. In the cytosol, cytochrome c associates with procaspase-9 and apoptosis protease activating factor-1 to form a signaling complex called the apoptosome.[20] Activated caspase-9 in turn promotes to the downstream activation of effector caspases and the induction of apoptosis. Mitochondrial permeability is controlled by the integration of pro-apoptotic and anti-apoptotic actions of the BCL-2 family of proteins.[21] Pro-apoptotic BAX and BAK form pores in the mitochondrial outer membrane, permitting cytochrome c release. This is opposed by anti-apoptotic family members including BCL-2, BCLX-L and Mcl-1. Cell fate is thus determined by the integration of signals mediating the activities of both pro- and anti-apoptotic BCL-2 family proteins. An upstream pro-apoptotic BCL-2 protein called BID is activated by enzymatic cleavage to truncated form (tBID), which orchestrates the activities of BAX and BAK to promote cytochrome c release. Cleavage of BID can be mediated by a number of proteases and is a characteristic of most intrinsic apoptosis pathways. Capase-8 can also cleave and activate BID, permitting cross-talk between the extrinsic and intrinsic apoptosis pathways.

A third pathway of caspase activation is mediated by granzyme B released from cytotoxic T lymphocytes (CTL) and NK cells. Co-release of perforin enables granzyme B to enter target cells where the substrates for its serine protease activity include caspase-3 and other caspases. The effect of granzyme B and certain other CTL granule enzymes is to produce the apoptotic death of target cells.

In addition to these classical cell death pathways a growing number of other pathways have been identified, some of which produce an apoptotic or necrotic modes of programmed cell death that may in different cases be dependent on or independent of caspase activity.[22,23] Most of these alternative pathways involve mitochondrial injury mediated by BAX or BAK. Major examples of such pathways include lysosomal apoptosis and pyroptosis. Lysosomal apoptosis occurs following lysosomal membrane permeabilization (LMP), which can be induced by oxidative stress, bile salts or chemotherapeutic drugs among other stimuli.[24,25] Lysosomes contain numerous proteases of diverse classes collectively called cathepsins. Many cathepsins are stored as proenzymes that become activated at low pH in phagolysosomes. Lysosomal enzymes accidentally released into the cytosol can trigger programmed cell death by directly damaging mitochondrial membranes or indirectly by cleaving BID and initiating apoptosis through BAX-mediated cytochrome c release.[26] Pyroptosis involves caspase-1-mediated activation of death-inducing enzymes including nucleases, but does not involve effector caspases.[27] Examples of macrophage pyroptosis has been reported following infection with *Francisella tularensis*, *Listeria monocytogenes* and *Shigella flexneri*.[28-30] Finally, extrinsic factors may promote apoptosis by directly causing injury to the outer mitochondrial membrane independent of BAX or BAK. One example of this is the VacA toxin produced by *Helicobacter pylori*.[31] VacA contains a mitochondrial localization motif and organizes the formation of pore in the outer mitochondrial membrane, triggering apoptosis.

Mtb ACTIVATES THE EXTRINSIC APOPTOSIS PATHWAY IN MACROPHAGES

In 1997 Keane et al.[32] first reported that infection of human alveolar macrophages by *Mtb* at a multiplicity of infection (MOI) of ~5 bacilli per cell was sufficient to induce classical, extrinsic apoptosis. This cell death was shown to be mediated by TNF-α in an autocrine/paracrine manner. It is well recognized that binding of *Mtb* by macrophages is a potent stimulus for TNF-α production. Naïve macrophages were shown to be insensitive to TNF-α-mediated apoptosis, but these cells became primed by the presence of live, intracellular mycobacteria to activate the TNFR1 death pathway.

Blocking phagocytosis with cytochalasin D or heat-inactivating *Mtb* prevented macrophage cytotoxicity. Another key finding in this study was that the attenuated *Mtb* strain H37Ra was a much more potent inducer of apoptosis than the virulent strain H37Rv despite comparable production of TNF-α. Subsequent studies confirmed the inverse correlation of *Mtb* virulence and the induction of classical apoptosis at low MOI.[33] This relationship is consistent with the hypothesis that TNF-α-mediated apoptosis of *Mtb*-infected macrophages is a defensive response, a model that was further supported by the discovery that virulent *Mtb* strains actively suppress apoptosis by interfering with TNF-α signaling and by upregulating the expression of anti-apoptotic Mcl-1.[34-36] The competing signals promoting activation and suppression of apoptosis in *Mtb*-infected macrophages is reminiscent of the pro-death and countervailing pro-survival signals in host cells infected with viruses.

CLASSICAL APOPTOSIS PROMOTES HOST DEFENSE IN TB

By denying infecting bacilli a protected intracellular environment for bacillary replication, TNF-α-mediated macrophage apoptosis presumably represents an innate defense that slows the increase of bacillary load following infection. However, this is not the only means by which apoptosis benefits the host in TB. Prior to the discovery of an intrinsic apoptosis response to *Mtb* infection, Molloy et al.[37] reported that inducing apoptosis of BCG-infected monocytes by exogenous drug treatment was accompanied by a reduction in bacillary viability. In contrast, when infected cells were made to undergo necrosis there was no reduction in BCG viability. The concept that apoptosis exerts a direct antimicrobial effect on intracellular mycobacteria was later supported by the demonstration that the viability of several apoptosis-inducing, attenuated mycobacterial strains (*Mtb* H37Ra, BCG and *M. kansasii*) was reduced as their host macrophages died.[33] In contrast, several virulent *Mtb* strains that provoked little or no macrophage apoptosis all demonstrated intracellular growth in culture. This paradigm has held up in several subsequent studies,[38-40] although the precise mechanism of the direct antimicrobial activity exerted in macrophages undergoing apoptosis has not been elucidated.

An indirect antimicrobial function of apoptosis was suggested by Fratazzi et al.[41] in experiments using an apoptosis-inducing strain of *M. avium*. Adding fresh, uninfected macrophages to cultures of infected macrophages that were undergoing apoptosis was associated with reduced mycobacterial viability but this was not seen if the initially infected macrophages were rendered necrotic. The authors showed that killing *M. avium* in this "add-back" culture system was contact-dependent, suggesting that it might involve engulfment by naïve macrophages of bacilli contained within apoptotic bodies. Similar results were reported by Lee et al.[42] using *Mtb* in a high-MOI challenge condition described below. In this system the initially infected macrophages exhibit features of apoptosis 3 hours post-infection. Adding naïve macrophages to the infected cells at this early time point resulted in a reduction of *Mtb* viability. If naïve macrophages were added at a later time point (18 hours) when the initially infected macrophages had progressed to necrosis, there was no evident negative impact on bacillary viability and growth of *Mtb* was observed. One explanation for these findings is that efferocytosis of apoptotic bodies harboring *Mtb* overcomes the typical restriction to phagosomal acidification and lysosome fusion orchestrated by the bacilli and instead delivers the microbes to an inhospitable, acidified phagolysosome.

While efferocytosis of *Mtb*-infected apoptotic corpses by macrophages might enhance host defense by killing bacilli, efferocytosis by dendritic cells (DC) makes a unique contribution to TB defense by promoting adaptive immunity. Schaible et al.[16] isolated apoptotic vesicles from BCG-infected macrophages and fed them to immature DC. Following uptake of these particles, the DC efficiently activated antigen-specific T cells including class I MHC restricted CD8+ T cells. This finding suggested that macrophage apoptosis could benefit the host by enhancing the priming of adaptive immunity which is critical to TB defense, and in particular by facilitating cross-priming of exogenous *Mtb* antigen to activate CTL. *In vivo* validation of this model was provided by the identifica-

tion of *Mtb* genes, including *nuoG* and *secA2*, required for the suppression of apoptosis by virulent *Mtb* strains.[40,43] Mutation of the *secA2* gene impairs secretion of superoxide dismutase and converts H37Rv to an apoptosis-inducing phenotype. Mice infected with the *secA2* mutant demonstrated increased priming of antigen-specific, MHC class I-restricted immunity. Vaccination of mice and guinea pigs with the *secA2* mutant induced protective immunity superior to BCG. Similarly, deletion of *nuoG* prevented *Mtb* from suppressing macrophage apoptosis and reduced its virulence *in vivo*.

It may be concluded that classical apoptosis of *Mtb*-infected macrophages is largely beneficial for the host and detrimental for the infecting bacilli. The capacity of *Mtb* to block TNF-α-mediated apoptosis is associated with virulence; it preserves the growth-supporting intracellular environment while limiting the antimicrobial effects of apoptosis and reducing the efficiency of priming adaptive immunity. The host has other means to cause apoptosis of infected macrophages, including the perforin/granzyme and Fas-mediated cell death triggered by CTL as well as other innate responses that will be described below. In the early stages of TB disease, when *Mtb* adopts a primarily intracellular lifestyle, the outcome of infection would thus appear to depend on the relative success of the host or the pathogen to stimulate apoptosis or promote the survival of infected macrophages.

Mtb USES PROGRAMMED CELL DEATH TO EXIT THE MACROPHAGE

Protecting the viability of host macrophages preserves an environment that supports intracellular *Mtb* replication. After infection at low MOI, virulent *Mtb* strains grow in macrophages by suppressing the innate TNF-α-dependent classical apoptosis pathway. In order to cause spreading infection, however, infecting bacilli must have some means to escape from the macrophage after reaching an optimal intracellular bacillary load and/or depleting metabolic resources provided by the host cell. Recently Lee et al.[42] reported that virulent strains induce a non-classical mode of macrophage cell death as way to exit the macrophage. This death is triggered when the intracellular bacillary load passes a threshold of approximately 20 bacteria per macrophage. It can be modeled by directly infecting macrophages at MOI $\geq$ 25 but it is also observed when macrophages infected at low MOI are cultured for several days, permitting the intracellular bacteria to multiply up to the threshold load for cytopathicity. Park et al.[44] infected macrophages at MOI 5 with several virulent *Mtb* strains having different rates of intracellular replication and found that those strains with the fastest growth rates killed their host cells after reaching ~15 to 22 colony-forming units per cell 6 days later. If *Mtb* replication was inhibited by treating the infected macrophages with IFN-γ, then macrophage viability was preserved even in those cells infected with the potentially cytopathic strains.

The death mode of heavily infected ("high-MOI") macrophages differs profoundly from the classical TNF-α-mediated and caspase-dependent death that occurs in human primary macrophages infected with attenuated bacillary strains at low MOI. In contrast to low-MOI apoptosis that is activated by attenuated bacillary strains and suppressed by virulent strains, cell death at high-MOI is most potently induced by virulent *Mtb* strains.[42] High-MOI cell death has faster kinetics than low-MOI apoptosis. Propidium-iodide (PI) positive necrotic cells are detectable within 6 hours of high-MOI challenge and there is nearly complete annihilation of infected macrophage cultures by 18 hours. This death mode initially has features of apoptosis, including nuclear condensation and externalization of phosphatidyl serine (PS) that is recognized by binding annexin-V. Unlike classical apoptosis there is minimal nuclear fragmentation or DNA cleavage and the apoptotic cells progress rapidly to secondary necrosis that releases bacilli from the confines of apoptotic cell envelopes. The term "apoptonecrosis" has been applied in this situation but is not favored by the Nomenclature Committee on Cell Death and will not be used here.[45] Rather, we will use the expression "high-MOI apoptosis" to indicate the death mode of heavily infected cells that begins with some typical characteristics of apoptosis but culminates in macrophage necrosis.

Experiments have excluded the involvement of

several established cell death signaling pathways in high-MOI apoptosis.[42] Evidence to date indicates no requirement for TNF-α, caspase activity, free radicals of oxygen or nitrogen, intracellular calcium flux or Toll-like receptor (TLR) signaling, all of which have been linked in different reports to *Mtb*-induced apoptosis. While the death signaling pathway activated by high intracellular *Mtb* load remains to be conclusively established, experiments suggest the possibility of lysosomal apoptosis. Macrophages heavily infected with *Mtb* were partially rescued from death by pre-treatment with cell-permeable inhibitors of cathepsins B and L.[42] Blocking lysosomal acidification with bafilomycin A also rescued heavily infected macrophages (J. Lee, manuscript in preparation), a finding consistent with a lysosomal death pathway since the activation of many cathepsins depends on lysosomal acidification. Considerations that support the involvement of lysosomal apoptosis in high-MOI cell death are that this death mode can operate independently of caspases,[31] and that *Mtb* infection has been shown to promote LMP.[46,47] Lysosomal membrane destabilization as a cause of high-MOI cell death can explain the characteristics of early apoptosis followed by rapid necrosis since the magnitude of LMP and amount of lysosomal enzymes released into the cytosol dictates whether this death mode has predominant features of apoptosis or necrosis.[48]

If high-MOI apoptosis functions as a pathway for *Mtb* to exit the macrophage, then it would be expected to lack the direct antimicrobial properties of classical TNF-α-mediated apoptosis. In this regard, Lee et al.[42] found that bacilli released from dying macrophages undergoing high-MOI apoptosis were not killed and were capable of subsequent extracellular growth. Whether the released bacilli are fully intact or suffer any transient compromise in their fitness remains to be determined. Interestingly, if naïve macrophages are added to high-MOI cultures 3 hours after the initial infection then there is fall in *Mtb* viability. The timing of this add-back experiment corresponds to the point when the infected cells demonstrate features of apoptosis (PS externalization, nuclear condensation) but have not become necrotic. In contrast to that result, if naïve macrophages are added to the infected cells at 18 hours, when necrosis is complete, then bacillary growth is not restricted. These data indicate that there is a window of vulnerability for infecting bacilli in the earliest stages of high-MOI apoptosis, but this is lost at some point after the bacteria are released into the extracellular environment from their necrotic host cells. The antimicrobial mechanism operating in these experiments has not been defined but the conditions appear similar to those described in add-back studies with *M. avium* where efferocytosis of apoptotic cells might deliver bacilli to a killing phagolysosome in the freshly added macrophages. Thus, while high-MOI apoptosis has features favorable for spreading infection there remains an opportunity for the host to limit the damage of poorly controlled bacillary replication.

OTHER DEATH MODES LINKED TO *Mtb* INFECTION

This review focuses on those death modes of *Mtb*-infected macrophages that have received the greatest amount of attention in published studies. However, it is important to note that several alternative mechanisms have been described. One closely related phenomenon is Fas-mediated apoptosis. Binding of FasL expressed on CTL to Fas expressed on *Mtb*-infected macrophages activates the classical extrinsic apoptosis pathway and, unsurprisingly, results in a bactericidal effect similar to TNF-α.[39] In keeping with the model that virulent bacilli benefit from apoptosis avoidance the surface expression of Fas was shown to be downregulated on *Mtb*-infected macrophages, making them insensitive to that death signaling pathway.

In a series of related manuscripts the laboratory of Dr. Mauricio Rojas has capitalized on macrophage-like cell lines derived from mouse strains that are resistant or sensitive to infection with BCG. In these studies, Dr. Rojas and colleagues describe infection-induced apoptosis that depends on the generation of nitric oxide and that is further regulated by IL-10 and TNF-α.[49-51] Of interest, there is differential susceptibility of cells derived from the resistant and sensitive hosts. A death signaling pathway operating downstream of TLR2 was reported to be stimulated by *Mtb* 19 kDa lipoprotein.[52,53] In these experiments, macro-

phage cytotoxicity was induced by inactivated bacteria and even by purified 19 kDa lipoprotein alone. Purinergic signaling by ATP ligation of the receptor P2X7R has been linked to the non-apoptotic death of *Mtb*-infected macrophages and to an antimicrobial mechanism that is distinct from that associated with classical, extrinsic apoptosis and which might involve the induction of autophagy.[54-56] Altogether, at least six discrete cell death pathways have been identified in macrophages ingesting or binding pathogenic mycobacteria. Differences in the conditions and mechanisms of infection-induced macrophage cell death have resulted in some confusion in the literature. It is reasonable to speculate that these varied results are not contradictory but rather reflect the potential for multiple pro-death (and pro-survival) signals to operate in this setting with particular pathways and outcomes predominating depending on variables including the host species, the source and differentiation state of the myeloid host cell, *in vitro* culture conditions, *Mtb* strain and the MOI.

THE APOPTOSIS/NECROSIS PARADIGM IN TB

The concept that one or more programmed cell death modes play an important role in TB pathogenesis is relatively new, but has gained traction in the TB research community. The various studies cited above suggest a paradigm where caspase-mediated apoptosis of *Mtb*-infected macrophages contributes to host defense while necrosis of these cells promotes spreading infection in active TB disease. This dichotomy was supported by Pan et al.[57] who investigated the host genomic basis for a strain of mice that are exquisitely susceptible to pulmonary TB. Susceptibility was mapped to a locus on chromosome 1 designated sst1 and a gene within that locus called Ipr1. Macrophages from mice with the susceptible sst1 allele were found to undergo predominant necrosis when infected with *Mtb in vitro*. This contrasted with the primarily apoptotic death of infected macrophages derived from sst1 resistant mice. A key observation in this work was that *in vivo* aerosol challenge of Sst1 susceptible mice was associated with massive pulmonary necrosis, while the histopathology of *Mtb*-infected Sst1 resistant mice showed the focal lesions typical of murine pulmonary TB, lacking any macroscopic necrosis.

In an unrelated study, Gan et al.[58] challenged mice at high MOI *in vivo* with virulent *Mtb* H37 Rv or attenuated H37Ra and showed by flow cytometry that the virulent strain induced higher rates of lung macrophage necrosis and dramatically depleted the resident alveolar macrophage population, while H37Ra caused significantly less necrosis and only partially reduced the resident macrophage population. Interestingly, the necrotic environment of the H37Rv-infected lung produced dramatically higher rates of neutrophil recruitment. While speculative at present, it is tempting to conclude that the newly described necrosis sensing receptor Mincle[59] is responsible for the pulmonary neutrophilia associated with H37Rv-induced macrophage necrosis.

The reports by Pan and Gan associate necrosis with TB susceptibility, while the previously referenced studies with *secA2* and *nuoG Mtb* mutants clearly demonstrate the enhancement of host defense when certain forms of macrophage apoptosis are promoted.[40,43] A model is emerging wherein the success of initial infection by *Mtb* depends on the pathogen's capacity to inhibit activation of the extrinsic apoptosis pathway in the host macrophage. The host has several alternative proapoptotic avenues available, including innate TNF-α death signaling as well as apoptosis induced by the adaptive CTL response through Fas signaling and by the action of perforin/granzyme. If apoptosis predominates then infection may be aborted and potentially cleared. However, if virulent bacilli succeed in suppressing caspase-mediated apoptosis of their host cells the bacilli may increase in number and subsequently activate a non-classical "high-MOI" death mode that progresses rapidly to necrosis. Macrophage necrosis promotes spreading infection to naïve macrophages and might also contribute to the macroscopic necrosis that characterizes advanced TB lesions. While adaptive immunity is thought to contribute to this destructive pathology, there is also evidence for an innate component[60] that could be reflective of high-MOI apoptosis occurring within TB granulomas. In this regard, Gil et al.[61] reported that necrosis of TB lesions in the lungs of infected

mice was related to bacterial load and could not be attributed to the function of any particular T cell subset or cytokine. It has recently been appreciated that *Mtb* imports and metabolizes cholesterol,[62] suggesting that necrosis could help create a cholesterol-rich milieu[63] geared to support extracellular bacillary survival, growth and transmission to the next human host.

Elucidating the interactions between *Mtb* and macrophages that culminate in the death of the infected cell (and in some circumstances the death of infecting bacilli) furthers our understanding of TB pathogenesis and could ultimately impact the diagnosis, treatment and prevention of TB. Apoptotic macrophages have been recovered by bronchoalveolar lavage of humans with TB.[64] While it is likely that dying cells will be present in the setting of diverse respiratory infections there is nonetheless potential that the presence and quantity of these cells might serve as a biomarker for active TB disease. In settings of poorly controlled mycobacterial replication one would predict an increased ratio of necrotic to apoptotic cells. Indeed, Sanchez et al.[65] reported that peripheral blood monocytes from TB patients demonstrated apoptosis and necrosis when infected *ex vivo* with H37Rv, while monocytes from healthy controls exhibited only apoptosis.

There is active investigation and development of drugs to regulate cell death responses for therapeutic indications including cancer, cardiovascular disease, neurodegenerative diseases, and aging among others.[66,67] Drugs that enhance classical apoptosis or those that inhibit the high-MOI death mode could have therapeutic value, while those with opposite effects could be detrimental to host defense in TB. The potential negative and positive impact of immune-modulating therapies on the risk for reactivation of latent TB infection and the treatment of active TB disease has been highlighted by the association of infliximab with TB susceptibility[68] and by reports of treating TB patients with recombinant IFN-γ,[69] respectively. It is clear that the evaluation of safety and efficacy for any novel drugs regulating cell death must consider their possible impact on the survival and death mode of *Mtb*-infected macrophages.

Perhaps the clearest near-term translational relevance of apoptosis in TB is in the field of vaccine development. Dr. Stefan Kaufmann and colleagues developed an experimental recombinant BCG strain that is presently in phase I clinical trials. This strain expresses the listerolysin O toxin (LLO) of *Listeria monocytogenes* and is also mutated to delete the mycobacterial *urease* gene.[70] The original concept was to promote the cross-presentation of BCG antigens on MHC class I through the action of LLO to permeabilize the mycobacterial vacuole and allow antigens to gain access to the cytosol. The *urease* mutation was designed to potentiate acidification in order to maximize the biological activity of LLO. Preclinical studies confirmed the potent immunogenicity of this candidate vaccine but also revealed that its potency is at least in part due to the induction of macrophage apoptosis. This discovery could lead to the development of even more effective vectors (or particles) building on the observation that apoptosis of infected macrophages enhances the priming and cross-priming of adaptive immunity. Future experiments may define the optimal characteristics of apoptotic bodies that promote antigen presentation and incorporate this knowledge into vaccine design.

CONCLUSION

Our understanding of TB pathobiology has become progressively deeper and more refined since the identification of *Mtb* as the etiologic agent of disease by Dr. Robert Koch in 1882. Incorporating concepts of macrophage programmed cell death following *Mtb* infection is a relatively recent addition to this knowledge base. We propose that following inhalation of infectious aerosols, *Mtb* bacilli invade lung macrophages and increase in number by intracellular replication. Host macrophages sense infection and may respond by undergoing TNF-α-mediated apoptosis and possibly through other cell death pathways. Apoptosis contributes to host defense by eliminating the niche for *Mtb* growth, by direct antimicrobial effects on intracellular bacilli, and by packaging *Mtb* bacilli and antigens in apoptotic bodies. The subsequent engulfment of these apoptotic bodies by newly recruited macrophages and dendritic cells promotes the eradication of

infection and the induction of the adaptive immune response. Virulent *Mtb* strains actively suppress apoptosis of the host macrophage to protect their replicative niche. After proliferating to a high intracellular load virulent bacilli trigger a necrotic mode of macrophage cell death, releasing them to infect new host cells and ultimately to grow as extracellular pathogens in necrotic cavities.

While much remains to be learned, the emerging model of macrophage apoptosis as beneficial to host defense and macrophage necrosis as a mechanism for spreading infection is becoming widely accepted. This is a complex topic, with the likely involvement of diverse and potentially competing cell death pathways and pro-survival signals that have different implications for the host and infecting pathogen. As with all new basic knowledge in biomedicine, the clinical significance of this work will only become clear as new information is incorporated into the design and evaluation of novel therapeutics. Given the central role that macrophage apoptosis plays in TB pathogenesis and to the induction of adaptive immunity, it is likely that new vaccines and potentially new therapies based on this knowledge will emerge in the coming decade.

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