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The role of macrophage cell death in tuberculosis

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Abstract

Studies of host responses to infection have traditionally focused on the direct antimicrobial activity of effector molecules (antibodies, complement, defensins, reactive oxygen and nitrogen intermediates) and immunocytes (macrophages, lymphocytes, and neutrophils among others). The discovery of the systems for programmed cell death of eukaryotic cells has revealed a unique role for this process in the complex interplay between microorganisms and their cellular targets or responding immunocytes. In particular, cells of the monocyte/macrophage lineage have been demonstrated to undergo apoptosis following intracellular infection with certain pathogens that are otherwise capable of surviving within the hostile environment of the phagosome or which can escape the phagosome. *Mycobacterium tuberculosis* is a prototypical 'intracellular parasite' of macrophages, and the direct induction of macrophage apoptosis by this organism has recently been reported from several laboratories. This paper reviews the current understanding of the mechanism and regulation of macrophage apoptosis in response to *M. tuberculosis* and examines the role this process plays in protective immunity and microbial virulence.

Keywords: macrophage; apoptosis; tuberculosis; *Mycobacterium tuberculosis*

Abbreviations: MTB, *Mycobacterium tuberculosis*; AMΦ, alveolar macrophage; TNF-α, tumor necrosis factor-α; TNFR, tumor necrosis factor receptor; CMI, cell-mediated immunity; DTH, delayed-type hypersensitivity; IFN-γ, interferon-γ; MDMΦ, monocyte-derived macrophage; IL-1β, interleukin-1β; LPS, lipopolysaccharide; iNOS, inducible oxyde synthase; CTL, cytotoxic T cell; TUNEL, terminal transferase digoxygenin-dUTP nick end-labeling; IL-10, interleukin 10; BCG, Bacille Calmette-Guerin.

Apoptosis in host defence

Host responses to pathogenic microorganisms present a complex series of layered defenses. These include mucosal barriers, engulfment by non-antigenic-specific phagocytes, and ultimately specific immunity initiated by antigen-presenting cells (dendritic cells, macrophages, and B lymphocytes) and orchestrated by antigen-specific T and B lymphocytes through cytokine signaling, cytotoxic T cell function, and antibody production. Clinical disease may occur both as a result of host cell destruction by invading pathogens, or as a consequence of the tissue destructive effects of the immune response. The traditional model of the host response to infection has focused on direct antimicrobial functions of immunocytes, but the discovery of programmed cell death has revealed a fascinating new aspect of the complex interaction between host and pathogen.

Apoptosis has been recognized as a component of protective host responses to virus infection for over a decade.[1,2] When target cells are hijacked by these intracellular parasites, apoptosis limits resources available for viral replication and reduces the injurious consequences of cellular necrosis. The importance of this mechanism in the host response to virus infection is highlighted by the numerous anti-apoptotic genes which have evolved in many viral genomes.[3] Certain bacterial and protozoan pathogens have evolved mechanisms to avoid elimination by cellular processes following phagocytosis, and these organisms are capable of survival and even replication within macrophages or other target cells. Infection by such intracellular pathogens presents the host with problem similar to that posed by viruses. It is not surprising then that apoptosis may also occur in this setting.

Target cell apoptosis in response to intracellular bacterial infection was first demonstrated with *Shigella flexneri* and *Bordatella pertussis*.[4,5] In the following half-decade apoptosis has been identified in response to a growing number of bacterial and protozoan pathogens (Table 1). In most of these cases the cells manifesting this response are of monocyte-macrophage lineage. Induction of macrophage apoptosis is typically observed following *in vitro* or *in vivo* infection, although in some cases pathogens appear to inhibit host cell apoptosis, indicating that cell fate depends on a balance of both pro- and anti-apoptotic signals that may originate from the cell or the pathogen.[6,7] This concept parallels the very complex interactions between cellular and viral effectors of cell death and survival.[8–11]

In the past decade the increasing incidence of clinically overt tuberculosis disease and the emergence of multi-drug resistance bacilli has stimulated clinical and basic research interest in this pathogen which is estimated to infect one third of the world's population.[12] Among the new insights arising from this research, macrophage apoptosis has recently been identified in response to *Mycobacterium tuberculosis* (MTB) infection. This paper will review the data

Table 1 Apoptosis and microbial pathogens

Intracellular bacteria	Host cell	Apoptosis	Reference
Shigella flexneri	Murine macrophage	Induction	57
Bordetella pertussis	Murine macrophage	Induction	4
Listeria monocytogenes	Murine hepatocyte and dendritic cell	Induction	58
	Murine macrophage	Induction of necrosis	59
Salmonella thyphimurium	Murine macrophage	Induction	60
Salmonella enterititidis	Monocyte	Induction	61
			57
Escherichia coli	Monocyte	Induction	61
			62
Actinobacillus actinomycetemcomitans	Murine macrophage	Induction	63
Psuedomonas aeruginosa	Human pro-monocyte	Induction	61
			64
Yersinia enterolitica	Murine macrophage	Induction	65
Staphylococcus aureus	Ephitelial cell monocyte	Induction	61
			66
Legionella pneumophila	Human pro-monocyte	Induction	67
Helicobacter pylori	Ephithelial	Induction	68
Mycobacterium tuberculosis	Human monocyte/macrophage and alveolar macrophage	Induction or inhibition	22
			23
			25
	Murine macrophage		29
Mycobacterium bovis BCG	Human monocyte	Induction or inhibition	24
			69
Leishmania donovani	Murine macrophage	Inhibition	27
Toxoplasma gondii	Lymphoblast	Inhibition of apoptosis induced by other stimuli	6
Chlamydia trachomatis	Epithelial Human pro-monocyte	Inhibition of apoptosis induced by other stimuli	7

describing this phenomenon and discuss the ways in which macrophage apoptosis might contribute to host defense against tuberculosis.

Macrophages and the immune response to tuberculosis

Tuberculosis infection is naturally acquired by inhalation. Those bacilli which manage to reach the alveolar compartment are phagocytosed by alveolar macrophages (AMΦ). If phagocytosis is followed by killing the bacteria, then the infection is eliminated. The AMΦ, however, may not necessarily mount an effective microbicidal response, permitting the establishment of persistent infection. Mycobacteria have evolved means to enhance their survival and replication within the macrophages by preventing maturation of the phagosomes which contain them. By inhibiting insertion of the membrane proton pump into its phagosome, mycobacteria prevent acidification of their intracellular environment. Furthermore, mycobacterial phagosomes can be restricted from fusing with the lysosomal compartment. In this way, intracellular bacilli are protected from potent host antimicrobial effector processes. Instead of functioning normally to kill internalized bacilli, the macrophage provides an environment suitable for bacterial replication.[13,14] Survival within the AMΦ may also serve to protect bacilli from other more effective host response mechanisms, and is likely to play a role in latency and persistence of tuberculosis infection. Latency is a characteristic feature of tuberculosis; in immunocompetent adults the initial infection and systemic dissemination are usually arrested without the development of clinical disease. In approximately 10% of infected individuals, dormant bacilli (believed to be located within macrophages) reactivate months or years later, resulting in clinically active tuberculosis. Thus, *M. tuberculosis* is a prototypical 'intracellular parasite' of macrophages and it is reasonable to postulate that defense mechanisms specific to intracellular pathogens may be an important component of host defense in tuberculosis.

If *M. tuberculosis* survives its initial encounter with the AMΦ, a granulomatous response may follow with the development of localized collections of epithelioid giant cells, macrophages, newly recruited monocytes, and lymphocytes. This reaction is antigen-independent and can be elicited experimentally by a variety of simple stimuli including systemic injection of mycobacterial cord factor.[15] Macrophage-pathogen interactions within the developing granuloma directly stimulate the release of several cytokines that have been linked to protection in animal models. Among them, tumor necrosis factor-α (TNF-α) is thought to play an important role and its expression in tuberculous granulomas has been directly demonstrated.[16] Antibody neutralization experiments, and studies of mice with targeted deletion of the TNF receptor p55 (TNFR1; 17) indicate that TNF-α is required for protection of the host. In the absence of TNF-R1, survival was dramatically reduced following infection with virulent *M. tuberculosis* and while granulomas formed in these animals, they contained more bacilli than controls and epithelioid cells were absent. Finally, there was more prominent lung tissue necrosis in the TNF-R1 deficient animals which has important

implications for the pathobiology of tuberculosis and disease transmission (*vide infra*).

While the granuloma provides a significant barrier to further spread of infection, infectious bacilli are not reliably eradicated by this response. Definitive antigen-specific host responses to tuberculosis include cell-mediated immunity (CMI) in which *M. tuberculosis*-reactive T cells activate macrophages to enhance killing of intracellular bacilli, and delayed-type hypersensitivity (DTH) characterized at the systemic level by tuberculin skin test reactivity and at the

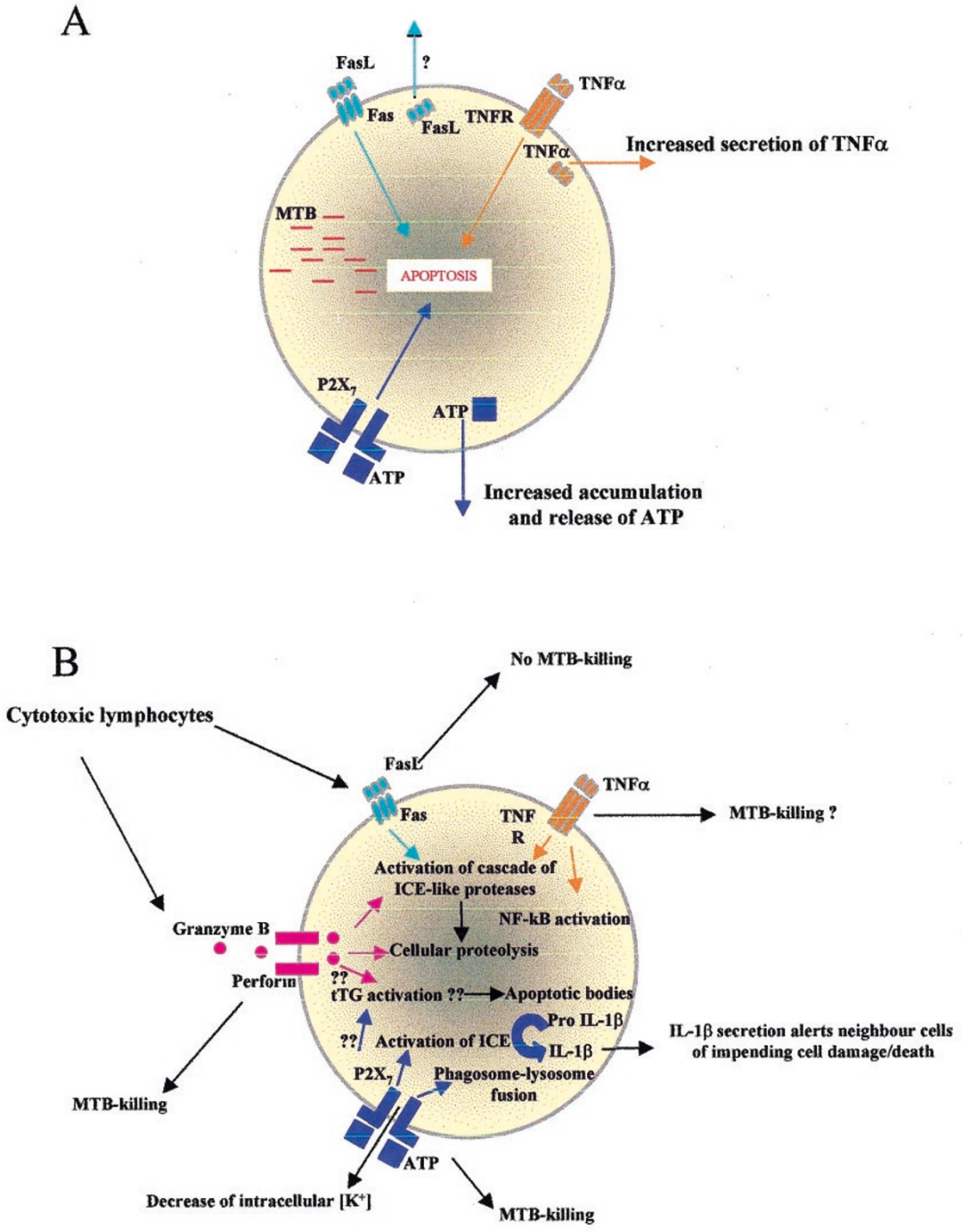

Figure 1 (**A**) Model for autocrine-paracrine induction of apoptosis by different pathways during infection of monocytes/macrophages by *Mycobacterium tuberculosis*. (**B**) Molecular mechanisms in the apoptosis of *Mycobacterium tuberculosis* infected-human macrophages. Activation of purinergic receptors by extracellular ATP and granule-dependent cytotoxicity was reported to be an effective mechanism for mycobacteria killing. On the contrary cytotoxicity of T cells mediated by Fas-FasL interaction had no effect on the viability of mycobacteria, while CD8+ T cells that lyse macrophages by granule dependent Fas-independent mechanism result in killing of the intracellular pathogen

local level by T cell-mediated killing of bacilli-laden macrophages.[18,19] It has been proposed that effective CMI contains infection with minimal tissue injury to the host, while DTH produces a more tissue-damaging response.[20] Macrophage activation in CMI is believed to be chiefly mediated by interferon-γ (IFN-γ) released from activated lymphocytes, although AMΦ themselves have recently been reported to produce and respond to endogenous IFN-γ following stimulation with *M. tuberculosis*.[21] In approximately 10% of infected individuals these antigen-specific responses are ultimately unable to control the infection. After a rather variable period of latency, progressive local pulmonary disease develops which is characterized by tissue destruction and cavity formation. Since man is the definitive host, lung tissue destruction is critical for the perpetuation of *M. tuberculosis*. Only by producing a necrotic infection with connection to the airway can infectious aerosols be produced enabling transmission of the microorganism to its next host.

Macrophage apoptosis in response to *M. tuberculosis* infection *in vitro*

Recent reports from several laboratories provide evidence that apoptosis is triggered by *M. tuberculosis* interaction with monocytes and macrophages including its definitive host cell, the AMΦ. Keane *et al*.[22] inoculated primary human AMΦ with *M. tuberculosis* strains H37Rv (virulent in mice) or H37Ra (attenuated). Macrophage apoptosis was observed with both strains, although H37Ra was a more potent inducer than H37Rv. This apoptosis was blocked by inhibition of endogenous TNF-α and accelerated by the addition of exogenous TNF-α, while the viability of uninfected macrophages was not affected by TNF-α. These data indicate that *M. tuberculosis* challenge in some way sensitizes AMΦ to autocrine and/or paracrine TNFα-mediated apoptosis. Apoptosis of primary human monocyte-derived macrophages (MDMΦ) following exposure of *M. tuberculosis* H37Rv *in vitro* was reported by Placido *et al*,[23] and Klingler *et al*[24] noted a similar response to H37Ra. The data of Placido and Keane both indicate that apoptosis requires challenge with live mycobacteria; heat-killed organisms had no effect on macrophage viability. This suggests that the signal which primes macrophages for apoptosis is generated from within the cell, rather than resulting from activation of cell surface receptors engaged by the bacteria.

The apoptotic response to mycobacteria may be restricted to mature macrophages. Durrbaum-Landmann *et al*[25] found that human monocytes isolated by counterflow centrifugation undergo spontaneous apoptosis over time in culture under unstimulated conditions. Apoptosis was reduced when monocytes were cultured with low numbers of H37Rv or with zymosan, but not latex beads. This result is consistent with earlier reports that monocytes undergo spontaneous apoptosis in the absence of growth factors, but can be rescued by activating agents including TNF-α, interleukin-1β (IL-1β), and bacterial lipopolysaccharide (LPS),[26] as well as by infection with *L. donovani*.[27] In contrast, differentiated macrophages lose their sensitivity to growth factor withdrawal but are susceptible to activation-induced apoptosis.[28] The regulation of programmed cell death in terminally differentiated, non-dividing, macrophages is clearly distinct from that of their cycling precursors. This distinction has important implications for the design of *in vitro* infection-induced apoptosis experiments using transformed cell lines or blood monocytes.

Apoptosis of murine macrophages following *in vitro* challenge with *M. tuberculosis* has also been identified. Rojas *et al*[29] tested murine macrophage cell lines congenic at the *Bcg/Nramp 1* gene, finding that B10R cells were more prone to apoptosis than B10S cells following exposure to live *M. tuberculosis* H37Rv. In contrast to primary human AMΦ, H37Ra did not stimulate apoptosis of the B10R cells in these studies. A role for NO^- and inducible nitric oxide synthase (iNOS) in B10 cell apoptosis was shown in experiments with aminoguanidine and nitroprusside, whereas human AMΦ apoptosis was previously found not to be affected by iNOS inhibitors.[22] However, apoptosis of B10R and B10S cells was inhibited by anti-TNF-α antibody, suggesting that the apoptotic mechanism for murine cells shares some similarities with the human macrophage response to mycobacterial infection. Of interest, treatment with mannose-capped lipoarabinomannan or LPS rescued murine B10R and B10S cells from apoptosis despite inducing NO^- production. While it is not certain that the responses of primary murine macrophages will be faithfully mirrored by tumor cell lines, these data suggest that apoptosis in response to intracellular infection by *M. tuberculosis* is common to both man and mouse. This is supported by induction of apoptosis in primary murine AMΦ by *M. tuberculosis* infection *in vitro* (J. Keane, unpublished observations).

Death signaling pathways in *M. tuberculosis*-infected macrophages

The data of Keane *et al*[22] indicate that TNF-α is a major initiator of apoptotic signaling for macrophages harboring intracellular mycobacteria. Infected macrophages become primed for killing by TNF-α, whereas uninfected macrophages are resistant to this effect. Several studies have noted that intracellular infection by *M. tuberculosis* and certain other intracellular bacteria can sensitize various cell types to TNF-α-mediated death[30–32] This suggests the existence of a common mechanism for the detection of viable intracellular bacilli that triggers a 'danger signal', priming the cell for elimination by TNF-α a cytokine that is almost universally induced by macrophage-pathogen interactions. The nature of this priming signal, whether it is of bacterial or cellular origin is a matter of speculation at this time. It is also unknown if this sensitization process occurs as a result of differential expression of TNFR1, or some downstream mechanism. Since TNFR1 mediates three different signaling pathways by differential association with various intracellular adaptor proteins (apoptosis via FADD, JNK activation, and NF-κB translocation;[33]) a number of control points are possible.

It is possible that simple engagement of cell surface receptors by mycobacterial cell wall components could suffice to trigger sensitization to TNF-α-mediated cell death, but this is unlikely since heat-killed *M. tuberculosis* has

been shown not to induce apoptosis.[22,23] Although extracellular signaling by LPS can induce AMΦ apoptosis,[34] the mechanism appears to be unrelated to that of mycobacterial infection. Apoptosis induced by LPS was unaffected by TNF-α but was accelerated by IFN-γ, whereas apoptosis induced by *M. tuberculosis* is accelerated by TNF-α but is not enhanced by IFN-γ[22] (and unpublished observations). Hayashi *et al*[35] reported that a sonicate of *M. avium* could induce apoptosis of human MDMΦ at concentrations of 300 μg/ml or greater, however, indicating that the potential for extracellular signals from mycobacteria to mediate cell death bears further investigation.

Death signaling receptor pathways other than TNF-R1 may also play a role in *M. tuberculosis*-induced macrophage apoptosis. The P2X purinergic receptor family are plasma membrane ligand-gated ion channels activated by ATP that are structurally related to *Caenorhabditis elegans* degenerin channels and to mammalian epithelial amiloride-sensitive sodium channels.[36] Mononuclear phagocytes express $P2X_7$ whose activation causes apoptosis in human MDMΦ and mouse microglial cells.[37,38] Recent data show that *M. tuberculosis*-infected MDMΦ increase the expression of $P2X_7$ receptors and that ATP accumulates and is released from these cells in the early phase of infection; this suggests an alternative autocrine/paracrine mechanism for the direct induction of macrophage apoptosis in response to infection (Mancino *et al*, unpublished observations).

Involvement of Fas in the macrophage death response to *M. tuberculosis* also bears consideration. Human MDMΦ have constitutive expression of Fas and undergo apoptosis if treated with soluble recombinant FasL.[39] However, Fas expression was down regulated by *M. tuberculosis* infection and there is no evidence that resting or infected MDMΦ express FasL. Thus, Fas-mediated death signaling may not play a role in the direct induction of apoptosis of infected macrophages prior to the recruitment of T cells to the lung when infection is controlled primarily by the innate immune activity of macrophages. In contrast, both Fas and purinergic receptor stimulation may be involved in cytotoxic T cell (CTL)-mediated killing of infected macrophages.

The fate of *M. tuberculosis*-infected macrophages may be determined not only by death signaling pathway activation, but also by the activity of downstream regulators of apoptosis. Evidence that nuclear translocation of NF-κB protects against TNF-α-mediated apoptosis[40,41] suggests a mechanism for regulating the apoptotic response of infected cells. Macrophage activation by *M. tuberculosis* activates a variety of signaling pathways, including NF-κB;[42] elevated levels of nuclear NF-κB would be expected to protect cells from infection-induced apoptosis while reduced levels would promote cell death. In this regard, a non-apoptosis-inducing mycobacterium (*M. smegmatis*) was recently found to cause apoptosis of target macrophages pre-treated with the proteasome inhibitor lactacystin (H. Remold, personal communication). The *bcl-2* gene family encodes dominant regulators of apoptotic cell death[43,44] that could also play a role in tuberculosis-induced apoptosis. Down-regulation of Bcl-2 protein expression was proposed by Klingler *et al*[24] as a factor regulating apoptosis of infected macrophages. They reported downregulation of Bcl-2 protein, but not Bax, following infection with Bacille Calmette-Guerin (BCG) or exposure to heat-killed *M. tuberculosis* H37Ra. The outcome of mycobacterial binding and phagocytosis might hinge on the relative strength or duration of pro- and anti-apoptotic signals affecting the relative levels of nuclear NF-κB activity as well as the intracytoplasmic ratio of Bcl-2 to Bax.

Macrophage apoptosis in tuberculosis *in vivo*

Macrophage apoptosis in response to mycobacterial infection would be of limited interest if it were only a phenomenon of *in vitro* culture. Several lines of evidence, however, indicate that apoptosis is a common event in tuberculosis infection *in vivo*, and that a significant proportion of macrophages at sites of granulomatous inflammation are susceptible. Placido *et al*[23] identified increased numbers of apoptotic AMΦ in bronchoalveolar lavage of tuberculosis patients, and even greater numbers in patients dually infected tuberculosis and HIV-1. This finding was confirmed by Klinger *et al*[24] in bronchoalveolar lavage studies comparing cells obtained from involved sites of the lung in patients with pulmonary tuberculosis to cells from uninvolved lobes of the lung in these same patients, and to normal control. Using *in situ* terminal transferase digoxygenin-dUTP nick end-labeling (TUNEL) the proportion of apoptotic cells was $14.8 \pm 1.9\%$, $4.3 \pm 0.9\%$, and $<1\%$ in these three groups, respectively. An early light microscopic survey of tuberculous granulomas reported the infrequent occurrence of cells with morphologic changes consistent with apoptosis.[45] Using the more sensitive *in situ* TUNEL method, Keane *et al*[22] examined lung sections from clinical cases of tuberculosis and estimated that $>50\%$ of cells in the periphery of granulomas were positive. Furthermore, Mancino *et al* (unpublished observations) found that in similar sections, typical tuberculous granulomata characterized by a core of caseous necrosis containing dying cells were surrounded by a cuff of apoptotic MΦ. The demonstration of increased TNF-α expression in these lesions[16] is consistent with its proposed role in macrophage apoptosis. The direct induction of macrophage apoptosis by *M. tuberculosis* is certainly only one of several pathways responsible for the TUNEL-positive cells demonstrated in clinical specimens. These tissues are virtually always obtained from patients with fully developed tuberculosis where the effects of CMI and DTH are also evident.

Host defense functions of macrophage apoptosis

While the evidence that apoptosis is a prominent macrophage response to tuberculosis infection is strong, it is much less clear what role it plays in the pathobiology of disease. It may be hypothesized that macrophage apoptosis benefits the infecting bacillus by depriving the host of an effective phagocyte without amplifying local inflammation that might be detrimental to bacterial survival. In this model, efficient

elimination of AMΦ by a mechanism that limits inflammation might interrupt or at least delay the induction of antigen-specific CMI and DTH responses. This hypothesis deserves testing, but an opposing model where macrophage apoptosis is predicted to benefit the host fits the pattern of responses to intracellular infection established for many other intracellular pathogens and provides a more satisfying explanation of the available data. The enhanced susceptibility to mycobacteria-induced apoptosis of murine B10R macrophages (derived from mice resistant to BCG), compared to B10S cells (from susceptible mice) supports a linkage between macrophage apoptosis and protection.[29] This hypothesis is further supported by the observation that the attenuated mycobacterial strains (e.g. H37Ra and BCG) are significantly more potent inducers of apoptosis than virulent strains such as H37Rv, Erdman, and wild type *M. bovis*[22] (and unpublished observations). Just as certain viruses produce anti-apoptotic factors, H37Rv was found to induce the shedding of soluble TNF-R2 (sTNF-R2) from infected AMΦ which inhibited the TNF-α death signal accounting, at least in part, for the reduced apoptosis caused by this strain as compared with H37Ra.[46]

Although *M. tuberculosis* growth may be inhibited after phagocytosis by AMΦ,[47] a microbicidal effect has not been demonstrated *in vitro* and it is clear that the bacillus continues to be matabolically active and may replicate within its phagosome.[48–50] Reports from several investigators indicate that apoptosis of infected macrophages, but not lysis, may effectively limit mycobacterial growth. Molloy *et al*[51] infected MDMΦ with BCG and subsequently treated the cells with either H_2O_2 to induce lysis or ATP to induce apoptosis. The viability of BCG was reduced only following treatment with ATP. These data were recently confirmed using MDMΦ infected with *M. tuberculosis* H37Rv (Mancino *et al*, unpublished observations). Induction of apoptosis of MTB-infected MDMΦ by pulsing with extracellular ATP enhanced the killing of intracellular mycobacteria, demonstrating a pivotal role of these receptors in the host defense against intracellular infection. The mechanism of bacterial growth inhibition by purinergic receptor activation is unknown, but effects on phagosome/lysosome fusion and vacuolar pH have been suggested,[51,52] (and Mancino *et al*. unpublished observations). Similarly, Oddo *et al*[39] demonstrated a reduction in mycobacterial viability when infected macrophages were stimulated with rsFasL. In contrast, Fas activation was found to have no effect on *M. avium* viability[53] while Stenger *et al*.[19] showed that CD1-restricted $CD4^-CR8^-$ cytotoxic T cells killed infected macrophages by a Fas-FasL interaction without killing the mycobacteria, whereas $CD8^+$ CTL killed target cells by a granule-dependent process which resulted in killing bacteria. These contrasting results, possibly explained by the very different experimental systems employed, leave open the question whether Fas-mediated apoptosis provides an antimicrobial effect similar to apoptosis induced by ATP.

In addition to any direct antimicrobial function of macrophage apoptosis, this process will significantly alter the interaction of infected macrophages with other host defense cells. Monocytes are actively recruited to sites of tuberculosis-induced inflammation *in vivo*,[54] where they may encounter *M. tuberculosis*-infected AMΦ undergoing apoptosis as well as bacilli contained in apoptotic bodies. The work of Fratazzi *et al*[55] indicate that mycobacteria presented to macrophages within apoptotic bodies may be handled differently than free bacilli, resulting in more potent suppression of bacterial growth. In these experiments, fresh uninfected autologous MDMΦ were added to cultures of MDMΦ previously innoculated with *M. avium* and undergoing apoptosis due to the mycobacterial infection. Uninfected macrophages were seen to adhere to the infected, apoptotic, cells and a reduction in bacterial growth of $>90\%$ was observed. In contrast, fresh MDMΦ added to cultures of infected MDMΦ which had undergone lysis failed to restrict bacterial growth. This result suggests that phagocytosis of bacilli packaged within apoptotic bodies might lead directly to lysosomal fusion, circumventing the interruption of phagosomal maturation seen when free *M. tuberculosis* bacilli are internalized.

In addition to limiting bacterial replication, macrophage apoptosis might also serve to limit tissue destruction in tuberculosis. If an orderly process of apoptosis predominates over cell lysis and tissue destruction,[56] the architecture of the lung will be preserved and the spread of infection to new hosts will be prevented. Man is the definitive host for *M. tuberculosis* and perpetuation of the bacteria requires it to establish a connection to the bronchial tree for aerosol transmission. In this regard, the lungs of mice with targeted deletion of TNF-R1 showed areas of necrosis in addition to granulomas following *M. tuberculosis* infection, whereas control mice had only granulomas without evidence of lung necrosis. In untreated human tuberculosis mortality and morbidity primarily result from lung damage and the residual pulmonary scarring following clinical tuberculosis contrast dramatically with minimal residual damage after even severe pneumonia due to routeine pathogens such as *Streptococcus pneumoniae*.

Conclusions

Alveolar macrophage apoptosis has now been demonstrated to be a common response to intracellular infection by *M. tuberculosis* both *in vitro* and *in vivo*. Experimental evidence supports a prominent role for TNF-α in this process; other death signaling receptor pathways such as $P2X_7$ and Fas may also participate. Host-protective functions for this response are likely to include the elimination of a protected intracellular environment conducive to bacterial replication or suitable for bacterial latency, as well as direct and indirect microbicidal actions. The former have been demonstrated experimentally but the mechanism for mycobacterial killing during apoptosis of infected macrophages is unknown. Experimental evidence suggests that indirect effects on mycobacterial replication are due to more effective handling of bacilli taken up when uninfected macrophages phagocytose apoptotic cells and debris. Additional roles for macrophage apoptosis in sparing tissue damage and signaling for monocyte and lymphocyte recruitment and activation are speculative but merit investigation.

It has been clearly demonstrated that not all strains of *M. tuberculosis* and related mycobacteria are equally potent inducers of macrophage apoptosis. Preliminary data indicate that *in vitro* infection with more virulent organisms results in significantly less macrophage cell death. The apparent inverse correlation between virulence and apoptosis is consistent with the more well understood relationship between viruses and their host cells where virus-encoded genes act to suppress the protective host cell apoptotic response. In the context of tuberculosis, this represents a new virulence associated phenotype whose mechanistic basis remains to be defined. Data indicate that in one example (H37Rv), the bacteria triggers a macrophage response (IL-10 production) that ultimately leads to inhibition of cell killing by TNF-α, but other mechanisms to either avoid triggering, or otherwise suppress, the apoptotic response may be identified as additional virulent strains are tested.

Macrophage apoptosis in response to tuberculosis infection has only recently been appreciated, and its implications for *in vitro* experimentation and for the pathobiology of tuberculosis disease remain to be explored. The choice of target cells for such experiments must be carefully considered since the available data indicate that primary monocytes, MDMΦ and AMΦ, and monocytic tumor cell lines, do not necessarily exhibit identical activities. While *in vitro* studies may provide new mechanistic insights, they will not be sufficient to determine how macrophage apoptosis functions in the context of the systemic host response to tuberculosis. Animal models are likely to contribute in this arena, but differences in immunology and pathology of human and murine tuberculosis must be considered in the interpretation of any result. An important first step will be to more completely define the similarities and differences in the response of primary murine and human macrophages to *M. tuberculosis* infection *in vitro*.

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