INT J TUBERC LUNG DIS 9(4):375–383

REVIEW ARTICLE

Role of mycobacteria-induced monocyte/macrophage apoptosis in the pathogenesis of human tuberculosis

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SUMMARY

Apoptosis is a physiological programmed cell death process whose dysregulation plays an important role in different human infectious diseases. An increasing number of intracellular pathogens are known to induce target cell apoptosis, while some other parasites inhibit it. Unlike necrosis, apoptosis is a silent immunological event occurring without inflammation. Infection-induced target cell apoptosis may be a successful strategy to eliminate pathogens and assure host survival. Conversely, apoptosis inhibition could represent an adaptive mechanism for pathogen survival, while it may be beneficial for the host to initiate an effective immune response.

The worldwide increase in tuberculosis has stimulated more research aimed at defining the interaction between *Mycobacterium tuberculosis* and the immune system. *M. tuberculosis* possesses sophisticated strategies to circumvent its fate within target monocytic cells. Apoptosis of alveolar macrophages and monocytes has been described as a consequence of *M. tuberculosis* infection. Moreover, the observation that mycobacterial lipoproteins activate macrophages through Toll-like receptor (TLR) 2 suggests that innate immune receptors contribute to defence against *M. tuberculosis*. There is evidence that TLR-induced apoptosis modulates inflammation and immune activation during *M. tuberculosis* infection. Finally, the role of apoptotic-infected cells as a source of microbial antigens for cross-priming of effector T-cells is also discussed.

KEY WORDS: apoptosis; tuberculosis; mycobacteria; monocytes; macrophages

THE RECENT worldwide increase in tuberculosis (TB) and the urgent need for a new and more effective vaccine have stimulated research aimed at defining the interaction between mycobacteria, host cells and the immune system. Cells of the monocytic lineage, and especially alveolar macrophages (AMs), are among the first host cells encountered by inhaled *Mycobacterium tuberculosis* deep in the lung.[1] Initially, resting phagocytes are poorly equipped to inhibit the growth of mycobacteria because of the pathogen's ability to take advantage of internalisation mechanisms. The host response ultimately results in the induction of cell-mediated immunity, which is relatively effective in containing the infection.[2–4] Caseating granuloma is the hallmark of an adequate immunological response whose organisation depends on intercellular contacts and on locally secreted cytokines and chemokines.[5,6]

Apoptosis is a physiological process of programmed cell death, involved during development and cell homeostasis. Apoptosis dysregulation plays an important role in different human infectious diseases.[7] To date, an increasing number of intracellular pathogens, including mycobacteria, have been identified to modulate apoptosis of target cells. Since the characterisation of morphological, molecular and metabolic features of apoptosis, many differences have been described in comparison with necrosis. Necrosis is a passive event leading to an inflammatory infiltrate involving many adjoining cells. Unlike necrosis, apoptosis is a silent, self-limiting process.[8,9] The quick removal of dying cells by neighbouring phagocytes avoids the spillage of intracellular contents causing inflammation and tissue destruction and prevents antigen (Ag) presentation. Figure 1 summarises the main differences between apoptosis and necrosis. However, more recent findings suggest that apoptosis modulates inflammation and immune activation, as discussed.

MYCOBACTERIA AND TARGET MACROPHAGE/MONOCYTE APOPTOSIS

Cells of the monocytic lineage are the main effectors against a broad spectrum of intracellular pathogens, including viruses, fungi, protozoa and mycobacteria.[10]

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Article submitted 1 February 2004. Final version accepted 27 July 2004.

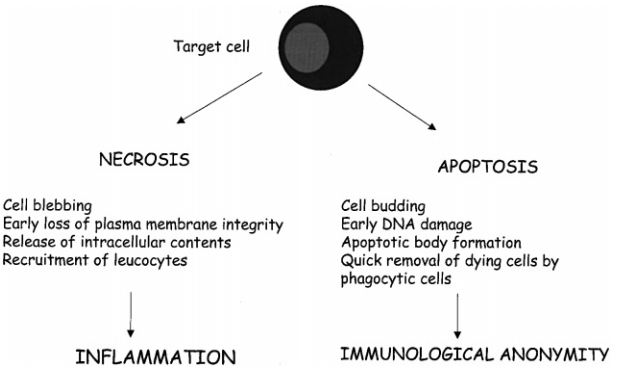


Figure 1 Necrosis vs. apoptosis: differences in cell morphology and cell death outcome.

To eliminate highly resistant pathogens, tissue macrophages and locally recruited monocytes must be primed by T-cell derived pro-inflammatory cytokines.[11] Activation of phagocytes is a crucial step for the host defence, but these cells are nevertheless dangerous because of their production of free radicals, lytic enzymes and inflammatory mediators. These molecules are responsible for extensive local tissue damage and for many local and systemic symptoms associated with inflammation. Apoptosis may eliminate no longer necessary activated cells and limit their destructive potential as inflammation wanes. The influence of mycobacteria on target cell apoptosis has received increasing attention over the last few years. Tissue macrophages are robust, long-living cells resistant to different apoptotic stimuli with few known mechanisms able to limit their activation.[12,13] *M. tuberculosis* is a potent apoptosis promoter of human macrophages.[14–17] Target cell apoptosis is mainly mediated by the expression of tumour necrosis factor-alpha (TNF-α) . This effect is enhanced by the participation of cytosolic phospholipase A2,[18] and is antagonised by the mannosylated lipoarabinomannan (ManLAM) through the alteration of Ca^{++} dependent cell signalling.[19] There is evidence that infection with the virulent *M. tuberculosis* H37Rv and Erdman strains produces a less significant effect on cell viability than infection with the attenuated strain H37Ra, or when *M. kansasii* or *M. bovis* bacille Calmette-Guérin (BCG) are used.[20,21] This result is mediated by the ability of virulent *M. tuberculosis* strains to induce the release by infected cells of soluble TNF-receptor 2 molecules which bind TNF-α, leading to its inactivation.[22] Figures 2 and 3 show examples of mycobacteria-induced apoptosis of human macrophages by means of different apoptosis detection procedures.

Unlike tissue macrophages, peripheral monocytes spontaneously undergo apoptosis when entering the inflamed site, unless given permission by inflammatory mediators or growth factors.[23,24] There is evidence that monocytes are less prone to undergoing apoptosis when infected in vitro with mycobacteria, unless high mycobacterial doses are used.[15] Indeed, low numbers of *M. tuberculosis* H37Rv prevent apoptosis of target human monocytes and promote the release of TNF-α.[25] In a similar fashion, *M. bovis* BCG increases the viability of infected monocytes, priming the secretion of TNF-α and inhibiting that of interleukin-10 (IL-10).[26]

GENETIC FACTORS AND MYCOBACTERIA-RELATED TARGET CELL APOPTOSIS

Growing evidence suggests that susceptibility or resistance to mycobacterial infection and target cell apoptosis is genetically determined. As shown in the animal model, the genetic background may account for target cell apoptosis/survival through the production of nitric oxide (NO) and the expression of pro-inflammatory and anti-inflammatory cytokines at the site of infection.[27] Resistant *M. tuberculosis*-infected murine macrophages (Bcg-r) undergo apoptosis and release high levels of NO and TNF-α, while *M. tuberculosis*-sensitive cells (Bcg-s) are resistant to apoptosis and produce lower levels of NO and TNF-α and high levels of IL-10.[28,29] Genetic factors may affect human susceptibility to TB, but no specific genes have yet been identified. The role of the human homologue of the *NRAMP1* gene, which influences susceptibility to TB infection in mice, is still unknown.[30,31] Bellamy et al. have suggested *NRAMP1* as a strong candidate for human TB because of the association of some *NRAMP1* polymorphisms with the clinical spectrum of the disease.[32] However, the data in the literature remain controversial and further studies are needed.[33–35]

EFFECT OF TARGET CELL APOPTOSIS ON MYCOBACTERIAL SURVIVAL

Mycobacteria display a wide spectrum of survival strategies to take advantage of internalisation mechanisms and escape the host immune response. Studies concerning the effect of target cell apoptosis on the viability of infecting bacilli have provided contrasting results. Lammas et al. have shown that the addition of adenosine triphosphate (ATP) to *M. tuberculosis*-infected human macrophages culminates with cell suicide and elimination of the bacilli.[36] There is evidence that ATP-mediated activation of purino-receptors promotes the phagosome-lysosome fusion and induces significant changes of the intraphagosome pH which are unsuitable for the growth of mycobacteria.[37–40]

While H_2O_2-induced apoptosis of *M. avium-intracellulare*-infected cells leads to the elimination of mycobacteria,[41] Fas-mediated cell death is not always coupled with their eradication. Indeed, while Fas ligand (FasL) induced apoptosis of human macrophages reduces the viability of infecting *M. tuberculosis*,[42,43] Fas-mediated T-cell killing of *M. tuberculosis*-infected cells has no effect on mycobacteria.[44,45]

Reactive nitrogen intermediates (RNI) are effective

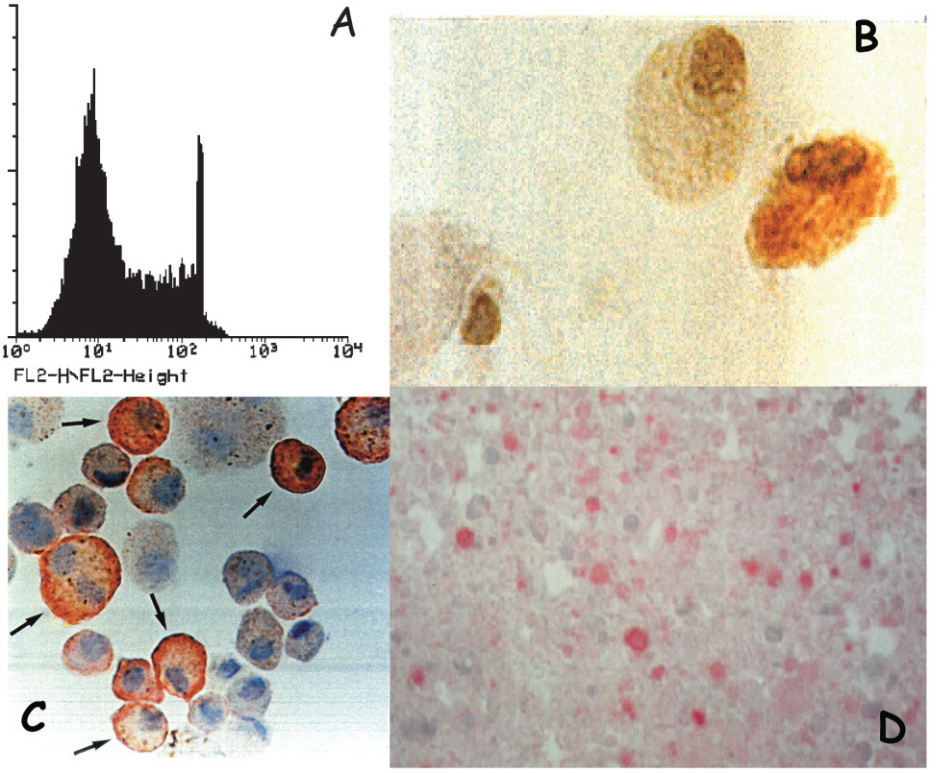
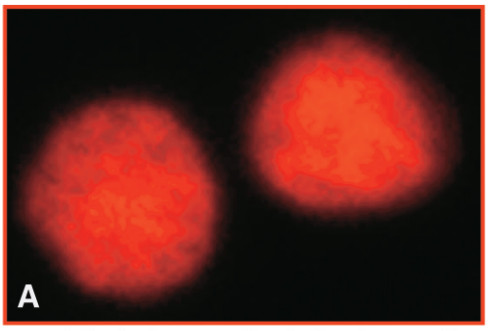


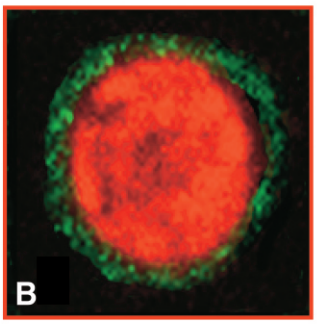
Figure 2 Examples of apoptotic cell detection in pulmonary TB patients. **A.** Flow cytometric analysis of propidium iodide stained apoptotic cells in bronchoalveolar lavage (BAL) fluid.

Demonstration of DNA fragmentation by TUNEL analysis in apoptotic alveolar macrophages (**B.** nuclear brown staining) and lung tissue (**D.** nuclear red staining) by immunochemistry.

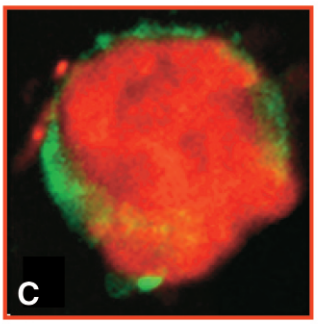
C. Cyto-plasmic protein aggregation in apoptotic cells by means of tissue transglutaminase (tTGase) activity.



Human uninfected monocyte-derived macrophages (MM)



Early apoptotic *M. tuberculosis* infected MM (MOI 20:1)



Late apoptotic *M.tuberculosis*-infected MM (MOI 20:1)

Figure 3 Annexin V membrane expression (green fluorescence) and nuclear iodide propidium (red fluorescence) staining by confocal laser microscopy are shown in uninfected and *M. tuberculosis*-infected human monocyte-derived macrophages (MM). Unlike uninfected MM (**A**), early apoptotic *M. tuberculosis*-infected cells are annexin V-positive (**B**). Additional nuclear chromatin condensation occurs in late dying infected MM (**C**) (magnification ×63). MOI = multiplicity of infection

mediators of host defence mechanisms against microbial agents. It is known that NO plays an essential antimycobacterial effect in the murine model of TB. The activation of the inducible NO synthase (iNOS), which leads to increased production of NO, correlates with host survival.[27–29] Findings in human models are controversial. Enhanced activity of iNOS has been detected in alveolar macrophages from TB patients,[46] and NO levels are increased in their respiratory samples.[47] However, the precise mechanism by which NO antagonises *M. tuberculosis* is not clear, but may involve disruption of DNA, proteins, signalling, and/or target cell apoptosis.[31,48]

Finally, the observation that control of mycobacterial growth may depend on target cell apoptosis comes from Frattazzi et al., who have shown that apoptotis, but not necrosis, of *M. avium*-infected human macrophages prevents mycobacteria from spreading and induces their growth inhibition by uninfected bystander phagocytes.[49] The complete inhibition of the intracellular growth of *M. avium* is achieved due to the synergic effect of IFN-γ and piconilic acid.[50]

APOPTOSIS AND GRANULOMA: IN VIVO FINDINGS

Apoptosis occurs in epithelioid granulomas,[51,52] but few data are currently available. Keane et al. found more than 50% apoptotic cells in the caseous areas of lung sections from clinical cases of TB.[14] However, further characterisation of apoptotic cells was not performed. More recently, the phenotype of dying cells has been analysed in biopsy specimens from patients with pulmonary and extra-pulmonary TB.[53] Interestingly, the majority of $CD68^+$ macrophages surrounding the caseous foci expressed the pro-apoptotic protein Bax and showed typical features of apoptosis, including Fas expression. Moreover, even adjacent memory T-cells, expressing both the pro-inflammatory cytokine, interferon-gamma (IFN-γ), and the death signals, Fas and FasL, are prone to undergoing apoptosis. These data suggest that at least Fas-FasL interaction may be involved in vivo in apoptosis of macrophages and T-cells in TB granulomas. However, as these findings cannot be demonstrated in situ, the role of other pro-apoptotic agents cannot be ruled out.

Finally, the observation that apoptotic cells are present in productive granulomas, but not in regressive lesions, suggests that apoptosis is an active process that modulates local cell-turnover, limiting tissue damage and spread of infection.[53]

TARGET CELL APOPTOSIS IN HIV-RELATED TUBERCULOSIS

There is wide evidence that *M. tuberculosis* impacts on human immunodeficiency virus (HIV) infection and that HIV promotes mycobacterial diseases. Clinical and experimental data suggest that the interaction of these two pathogens leads to accelerated disease progression in co-infected patients.[54–58] Recent epidemiological studies have estimated that HIV infection increases the 2–23% lifetime risk of reactivation of latent TB to 5–10% per year.[59,60] HIV infection is associated with an inadequately increased rate of T-cell apoptosis due to chronic immune activation. Our data showing a higher ex vivo rate of apoptosis of monocytes and AM in *M. tuberculosis*-HIV co-infected patients compared to HIV-negative TB patients support the existence of a synergic interaction between these two pathogens.[15] Moreover, the observation that apoptosis correlated with disease severity suggests that death of target cells is an emergency strategy of the host to eliminate infected reservoirs. Finally, spontaneous apoptosis of mononuclear phacocytes and of Ag-specific $CD4^+$ T-cells occurs in patients affected by TB pleuritis at the site of infection.[61]

APOPTOSIS, INFLAMMATION AND IMMUNITY: THE ROLE OF MYCOBACTERIAL LIPOPROTEINS

More recent data have suggested that apoptosis modulates inflammation and immune responses. The first observation that infection-induced target cell apoptosis is linked to inflammation comes from Zychlinsky et al., who found that infection of macrophages by *Shigella flexneri* triggers target cell suicide and promotes the cleavage of pro-IL-1β into a mature isoform. The release of the intracellular store of IL-1β initiates inflammation through the recruitment of neutrophils into the infected site.[62]

The recent finding that pathogen-associated molecular patterns (PAMPs), including the bacterial cell wall associated lipoproteins, are ligands of human Toll-like receptors (TLRs) opens new perspectives for understanding the host pathogen interaction.[63–65] Signalling through TLRs influences the innate immune response to infection by upregulation of immunomodulatory molecules and secretion of antibacterial effectors, which may lead to the development of a Th1-biased response.[66,67] It has been shown that the 19-kDa lipoprotein of *M. tuberculosis* activates TLR-2, leading to the induction of target cell apoptosis in association with the expression of pro-inflammatory mediators, as shown in Figure 4.[68] Ciaramella et al. have also shown that *M. tuberculosis*-induced apoptosis of human monocytes is coupled with an increased release of IL-1. This effect is inhibited by the neutralisation of the mycobacterial 19-kDa lipoprotein.[69,70] However, the observation that chronic stimulation of TLR2 by *M. tuberculosis* 19-kDa lipoprotein inhibits IFN-γ dependent induction of major histocompatibility complex (MHC) class II expression and Ag presentation in a mouse model, allowing *M. tuberculosis* to evade detection by $CD4^+$ T-lymphocytes, provides

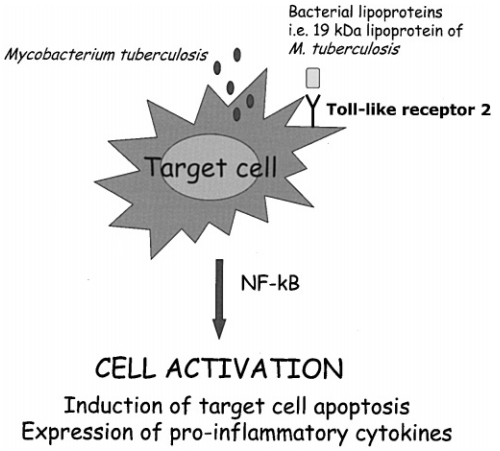


Figure 4 Apoptosis and inflammation: role of mycobacterial lipoproteins. NF = nuclear factor.

a mechanism whereby mycobacteria may persist undisturbed in host cells.[71] Similar results have also been shown in 19-kDa stimulated human macrophages.[72] In addition, the 19-kDa dependent dysregulation of the MHC-class I processing pathway, through the inhibition of phagosome maturation and Ag degradation, with a resulting decrease in peptide regurgitation, further contributes to immune evasion.[73]

INFECTION-INDUCED APOPTOSIS AND CROSS-PRIMING: CONSEQUENCES FOR T-CELL IMMUNITY

Yrlid et al. observed that apoptosis induction by bacterial infection of macrophages may not be a quiescent death that allows pathogens to escape recognition by the immune system, but contributes to an antimicrobial immune response on engulfment by bystander cells.[74] They showed that antigens derived from *Salmonella*-infected macrophages undergoing apoptosis were presented on MHC-class II and MHC-class I molecules by bystander dendritic cells (DCs) after internalisation of apoptotic cells. It was previously shown that human DCs are able to efficiently present Ags derived from apoptotic cells and stimulate class-I-restricted cytotoxic $CD8^+$ T lymphocytes.[75] Apoptotic infected cells may efficiently provide antigen for cross-presentation in many different types of infection. Cross-priming would be beneficial to the host because it results in $CD8^+$ T-cell activation in those cases in which the pathogen does not directly infect an antigen presenting cell (APC) or when the pathogen inhibits antigen presentation.[76,77] Mycobacteria-induced apoptosis of macrophages causes the release of apoptotic vesicles that carry mycobacterial antigens to uninfected bystander DCs. The engulfment of these vesicles, containing both proteins and glycolipids, is essential for antigen presentation, through MHC-class I and CD1b molecules, to efficiently prime $CD8^+$ T-cells (Figure 5).[78]

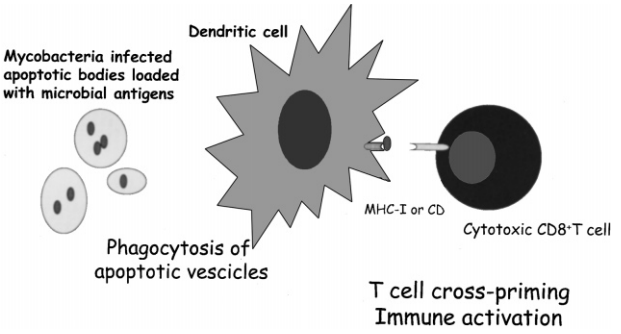


Figure 5 Dendritic cell mediated cross-priming of effector T $CD8^+$ cells by microbial antigens from phagocytosed mycobacteria-infected apoptotic cells. MHC = major histocompatibility complex.

CONCLUSIONS

Modulation of target cell apoptosis in response to infection with mycobacteria and its effect on pathogen viability are not completely understood. Mycobacteria possess both apoptotic and anti-apoptotic signals that suggest the existence of an inverse correlation between pathogen virulence and its ability to induce target cell apoptosis. Moreover, the host cell features, the cytokine pattern in the culture media and the host genetic background are additional factors able to modulate the outcome of the infection. The question as to whether target cell apoptosis is beneficial to the host or rather to the pathogen still remains open. As shown in Figure 6, killing of target cells may favour mycobacteria by reducing the number of efficient APCs and interfere with the induction of an appropriate cell-mediated immune response. Indeed, if there are fewer target cells serving as protected pathogen sanctuaries, the number of surviving bacilli will decrease, leading to resolution of the infection. Conversely, prevention of host cell apoptosis may be considered as an economy strategy of the immune system to conserve activated APC to generate an effective T-cell memory. This mechanism may be useful to ultimately eliminate both the infected cell and the pathogen.

However, as previously discussed, the more recent observations suggesting that infection-induced apoptosis modulates inflammation and that immune responses provide new mechanisms of host-pathogen interaction should be further clarified. In conclusion, additional efforts are required to obtain insights into how apoptosis may influence the pathogenesis of TB. A better understanding of apoptosis, of its related mechanisms and of its ability to modulate the immune response may offer new strategies to decipher the complex cross-talk of mycobacteria with humans.

Acknowledgements

This work has been supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica and by the Istituto Superiore di Sanità.

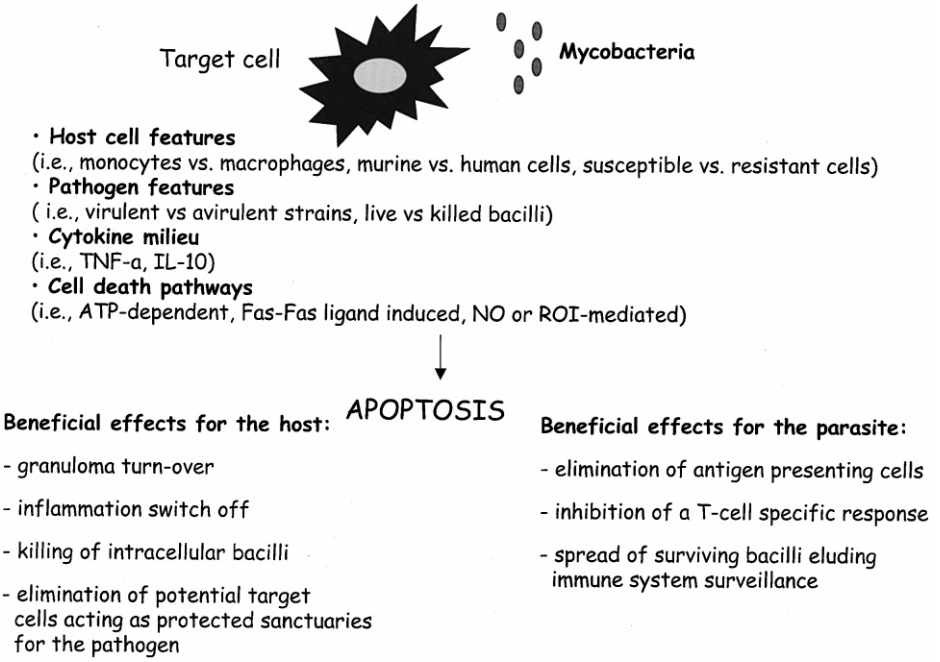


Figure 6 Modulation of mycobacteria-induced target cell apoptosis. Consequences for the host and the pathogen. TNF = tumour necrosis factor; IL = interleukin; ATP = adenosine triphosphate; NO = nitric oxide; ROI = reactive oxigen intermediates.

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RÉSUMÉ

L'apoptose est un processus de mort cellulaire programmé physiologiquement dont les troubles jouent un rôle important dans les différentes maladies infectieuses humaines. On sait qu'un nombre croissant d'agents pathogènes intracellulaires induisent une apoptose des cellules-cible alors que quelques autres parasites l'inhibent. A l'opposé de la nécrose, l'apoptose est un événement immunologique silencieux survenant sans inflammation. L'apoptose induite par l'infection au niveau de cellules-cible peut être une stratégie couronnée de succès pour éliminer le pathogène et assurer la survie de l'hôte. A l'inverse, l'inhibition de l'apoptose peut représenter un mécanisme d'adaptation assurant la survie de l'agent pathogène alors qu'elle peut être utile à l'hôte pour l'initiation d'une réponse immune efficace.

L'augmentation mondiale de la tuberculose a stimulé de nombreuses recherches visant à définir l'interaction entre *Mycobacterium tuberculosis* et le système immunitaire. *M. tuberculosis* possède des stratégies sophistiquées pour circonvenir son destin au sein des cellules monocytaires cible. L'apoptose des macrophages alvéolaires et des monocytes a été décrite comme une conséquence de l'infection *M. tuberculosis*. De plus, l'observation selon laquelle les lipoprotéines mycobactériennes activent les macrophages à travers un récepteur «toll-like» (TLR) 2 suggère que les récepteurs de la réponse innée contribuent à la défense contre *M. tuberculosis*. Il est prouvé que l'apoptose induite par TLR module l'inflammation et l'activation immunitaire au cours de l'infection *M. tuberculosis*. Finalement, le rôle des cellules infectées en apoptose comme source d'antigènes microbiens pour l'amorçage croisé des cellules T effectrices est également mentionné.

RESUMEN

La apoptosis es un mecanismo fisiológico de muerte celular programada, cuya disfunción juega un papel primordial en diferentes enfermedades infecciosas en el hombre. Cada vez se conocen más patógenos intracelulares que inducen la apoptosis de sus células diana y otros que la inhiben. A diferencia de la necrosis, la apoptosis es un evento inmunológico silencioso que ocurre sin inflamación. La apoptosis de la célula diana, inducida por la infección, puede corresponder a una estrategia exitosa para eliminar el patógeno y garantizar la supervivencia del huésped. Por el contrario, la inhibición de la apoptosis podría representar un mecanismo de adaptación para la supervivencia del patógeno, y al mismo tiempo favorecer al huésped facilitando el comienzo de una respuesta inmunitaria eficaz.

El incremento mundial de la tuberculosis ha estimulado la investigación que busca definir la interacción entre *Mycobacterium tuberculosis* y el sistema inmunitario. *M. tuberculosis* dispone de estrategias sofisticadas para evitar su destino dentro de las células diana mono-

cíticas. Se ha descrito la apoptosis de macrófagos alveolares y de monocitos como consecuencia de la infección por *M. tuberculosis*. Además, la observación de que las lipoproteínas micobacterianas activan los macrófagos a través del receptor Toll en drosófila (TLR-2) indica la existencia de inmunorreceptores innatos que contribuyen a la defensa contra *M. tuberculosis*. Existen indicios de que la apoptosis inducida por el TLR-2 regula la inflamación y la activación inmunitaria durante la infección por *M. tuberculosis*. Por último, también se discute el papel de las células apoptóticas infectadas, como fuente de antígenos micobacterianos para la sensibilización cruzada de las células T efectoras.