… differences, within and between countries, in income levels, opportunities, health status, access to care, and life expectancy are greater today than at any time in recent history.

Dr Margaret Chan

…and in my lived-in loneliness I found time to think about hope: some day our lives will not be, as Hobbes said, just short, brutish and nasty?’

_Jonas’ Report_
Jose Emilio Pacheco

_Deep Waters_
Manuel Mendive
Oil on canvas; 260 × 290 cm
INTRODUCTION

Different variants of cell death are present in TB, influencing the infectious process and the characteristics of the immune response to MTB (1–3). Apoptosis, one of the processes involved in cell death, plays an important role in TB and offers the possibility of applications related with prevention, diagnosis and therapeutics (1–3). During apoptosis, the cytoplasmic contents of dying cells including the intracellular pathogens are packaged into membrane bound vesicles called apoptotic bodies which elicit signals that promote engulfment and clearance of dead cells by specific phagocytes (4). Thus apoptosis, which is a conserved cell death mechanism in animals and plants, appears as one of the effective host innate immune defense mechanism to counter intracellular pathogens (5). Apoptosis of infected cells benefits the host by reducing bacterial viability. The apoptotic bodies containing the MTB pathogen serve as a source of bacterial antigens which induce pathogen specific T-cell immunity that potentially contribute to protection of the host from the bacteria (1, 3). This chapter briefly summarizes some of the important aspects of apoptosis in the immune response against TB and its current applications in vaccine development. For a more detailed insight into these aspects several excellent reviews are available (1–3, 6–9).

APOPTOSIS AND MTB INFECTION

MTB has been shown to modulate apoptosis in the host cells by inducing or inhibiting this cell death mechanism (1). MTB induces apoptosis in infected macrophages in a process mediated mainly by TNFα (1, 3, 10). It has been shown that in the presence of intracellular mycobacteria, macrophages undergo TNFα-mediated apoptosis by activating the TNF-receptor 1 (TNFR1) death pathway (10). In fact apoptosis induced by virulent strains of MTB has been reported for primary human alveolar macrophages (11, 12), monocyte derived macrophages obtained from human blood (13) and bone marrow derived murine macrophages (14). For example, the virulent MTB-H37Rv strain causes induction of apoptosis in infected
primary alveolar macrophages in vitro (10). Host factors, particularly eicosanoids seem to regulate apoptosis of MTB-infected cells (15). Recent studies showed that apoptosis of MTB-infected macrophages results in restriction of early bacterial growth and serves as an excellent means of antigen presentation that aids in initiation of T-cell mediated acquired immunity (1, 3, 15, 16). On the other hand, MTB employs multiple mechanisms to inhibit apoptosis of infected macrophages in order to survive and to establish the infection (1, 3). Among such mechanisms are interference with TNFα-signalling (3), over expression of anti-apoptotic Mcl-1 molecule (17) and the suppressive effect of MTB genes such as nuoG (18), pknE (19), and secA2 (20).

Virulent mycobacteria including MTB-H37Rv, MTB-Erdman and M. bovis significantly reduced TNFα-mediated apoptosis in primary human alveolar macrophages compared to non-virulent species such as BCG, MTB-H37Ra and M. kansasii (21). Virulent MTB-H37Rv also markedly inhibits FasL-mediated apoptosis of infected macrophages compared to wild type or MTB-H37Ra (22). These findings suggest a negative correlation between MTB virulence and infection-induced apoptosis of host macrophages, which is consistent with the notion that TNFα-mediated apoptosis of MTB-infected macrophages is a defensive response of the host and that such strains actively inhibits/down-regulates apoptosis by interfering with TNFα signaling (3) and inducing over expression of the anti-apoptotic protein, Mcl-1 (17).

Three distinct genes, nuoG, pknE and secA2, of MTB were identified as anti-apoptotic factors as they downregulate induction of apoptosis of the non-virulent M. smegmatis and M. kansasii (1). Over expression of nuoG gene, which encodes NuoG subunit of type I NADH (nicotinamide adenine dinucleotide)-dehydrogenase in MTB, inhibits apoptosis of infected macrophages whereas deletion of this gene enhances the induction of apoptosis of infected cells and reduces the virulence of MTB (18). The pknE gene, which encodes a protein kinase induced during nitric oxide (NO) stress, is a virulence factor that induces the inhibition of apoptosis, and the deletion mutant of pknE enables MTB to induce a higher level of apoptosis of infected human macrophages (19). Mutation of secA2 gene is involved in impaired secretion of superoxide dismutase A, and secA2 mutant of virulent MTB-H37Rv loses its virulence and induces higher level of macrophages apoptosis upon infection compared to wild type MTB (20). Mice challenged with secA2 mutant of MTB display an increase in antigen-specific MHC class I-restricted immunity (8). Therefore, while MTB have been reported to induce apoptosis in vitro, which seem to favor host protection, the virulent pathogens also set in motion anti-apoptotic mechanisms that inhibit apoptosis in order to escape immune recognition and continue to infect nearby cells, thereby compromising host defenses (1, 3, 23–25).
Apoptosis promotes the elimination of infected cells while minimizing tissue destruction (26–28). Supporting this notion is the presence of multiple apoptotic cells in resolving granulomas (28), in contrast to the presence of abundant necrosis in the centre of unresolved granuloma (29). Apoptosis of infected cells favours cross priming of mycobacterial antigens by uptake of apoptotic vesicles by DCs, thus improving cell-mediated immune responses (30). On the other hand, the induction of necrosis, a characteristic of the most virulent strains induce tissue damage and dissemination of the infection (1, 3). The net result of infection depends on the host’s ability to mediate apoptosis and pathogen’s ability to restrict this process and to survive within host macrophages. Basically, the more virulent the MTB strain the higher its inhibitory effect on apoptosis (1, 3).

APOPTOSIS AND TB VACCINE DEVELOPMENT

Considering the important role of apoptosis in the immune response against TB, the development of vaccine candidates which induce apoptosis to increase the protection against TB have been reported (31–33). Recombinant BCG deficient in urease-C and expressing the perforin, listerolysin (Hly) of *Listeria monocytogenes* demonstrated superior protective capability than BCG upon aerosol challenge with MTB, including the Beijing/W genotype family, which has been associated with strong induction of apoptosis in infected cells (31). A pro-apoptotic mutant of MTB with an inactivation of the *SecA2* gene demonstrated increased priming of antigen specific CD8+ T-cell in vivo and induced enhanced protection in mice and guinea pigs after challenge with MTB compared to BCG vaccination (32). An additional mutation of this strain conferring lysine auxotrophy retains the pro-apoptotic nature and augmented CD8+ T-cell stimulatory effect with an improved safety profile (33). DNA vaccination with a construct encoding Ag85A antigen of MTB with caspase-3 induced superior protection after MTB challenge compared with immunization with a DNA construct expressing only the antigen (34). However, a recent study evaluating an attenuated MTB vaccine strain did not find any correlation between the protective capacity of the MTB strain and induction of apoptosis (35). Several growth or cellular factors including the type of culture media used for mycobacterial growth and maturity of the host’s immune system may influence the capacity to induce apoptosis (36, 37). Nonetheless, there is growing evidence that induction of apoptosis during an immune response against infection, including infection with mycobacteria, may be an important strategy for the development of new generation vaccines to enhance their protective capacity against MTB.
REFERENCES


