Macrophage apoptosis in host immunity to mycobacterial infections

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Abstract

Macrophage apoptosis occurs within the granuloma, which is essential for successful immunity to tuberculosis. In vitro macrophage apoptosis is associated with the killing of intracellular Mycobacterium tuberculosis. A greater understanding of these observations will lead to new immunotherapies and improved vaccine design. The relevant apoptotic stimuli, the anti-mycobacterial mechanisms that they stimulate and their physiological relevance are reviewed in this paper.

Introduction

Tuberculosis is the most important mycobacterial infection in humans, being the second major infectious cause of death and killing two million people every year [1]. However, many more are infected, approx. one-third of the world's population. Fortunately, 90% of those infected remain healthy by either eradicating the Mycobacterium tuberculosis or holding it dormant throughout their life [2]. If we are to design effective vaccines and improve treatments, we need to understand both successful host immunity and how it fails when disease occurs.

Apoptosis and the granuloma

The major histological feature of mycobacterial infection is the granuloma. This consists of a core of macrophages surrounded by lymphocytes. Macrophages infected with mycobacteria promote Th1 (T helper 1) lymphocyte activity by the release of IL-12 (interleukin-12) [3], IL-18 [4] and IL-23 [5]. These Th1 lymphocytes then drive macrophage activation by the release of interferon-γ [6] and TNFα (tumour necrosis factor α) [7]. Whether eradication or disease ensues depends on the balance between macrophage mycobacterial activity and the virulence factors of the mycobacterium.

The granuloma is a dynamic entity, with macrophages dying and being replaced by newly activated monocytes. Similarly, lymphocytes are continuously replaced as cell-mediated immunity improves the specificity of cells. Both apoptotic macrophages and lymphocytes can be seen within the granuloma [8]. Tuberculous granuloma, as opposed to the granuloma seen in other diseases, often have a core of necrotic cells and are termed caseating. It is within these caseating granuloma, bordering the necrotic area, that high levels of apoptotic macrophages are seen [9].

Apoptosis can be triggered by either the intrinsic pathway, involving mitochondrial release of cytochrome c and activation of caspase 9, or the extrinsic pathway, involving the stimulation of death receptors expressed on the cell surface and activation of caspase 8. Macrophages within tuberculous granuloma express the pro-apoptotic protein Bax, but not the anti-apoptotic protein Bcl2 [10]. These proteins are a part of the intrinsic apoptotic pathway regulated by cell stress, such as hypoxia that occurs within the granuloma. In addition, high levels of both the cytokine TNFα [11] and Fas ligand expression on neighbouring lymphocytes [10] are present. These stimulate the classical death receptors p55 TNFR (TNF receptor) and Fas.

A combination of these classical stimuli, and other stimuli mentioned later, are presumed responsible for the high levels of apoptosis seen within tuberculous granuloma.

Benefits of macrophage apoptosis to the host

Macrophages undergoing apoptosis kill intracellular mycobacteria, whereas those undergoing necrosis do not. This has been demonstrated using a number of apoptotic stimuli, e.g. Fas ligand [12], TNFα [12], picolinic acid [13], ATP [14] and the mycobacterial 19 kDa lipoprotein [15]. Whether the same mycobacterialidial mechanisms are activated by the different stimuli is unknown.

p55 TNFR and Fas are members of the TNFR family. Their apoptosis-signalling pathways are well characterized, involving the interaction of intracellular death domain regions with the TNFR-associated death domain that interacts with the Fas-associated death domain to activate caspase 8. The importance of TNFα in host immunity to tuberculosis is demonstrated by the susceptibility of p55 TNFR gene-disrupted mice [7] and human patients treated with TNFα antagonists [16]. The TNFα-induced mycobacterial mechanism partly involves the production of nitrogen radicals in murine models [17] and is unknown in humans. Little is known about how Fas induces mycobacterial killing.

The 19 kDa lipoprotein, along with lipoaribomannan and phosphatidylinositol mannan, is a pathogen-associated molecular pattern expressed by M. tuberculosis, which activates TLR-2 (Toll-like receptor 2) [18]. At least ten
different TLRs are found on macrophages and dendritic cells, the antigen-presenting cells. They not only play a role in innate immunity, but also, through influencing the cytokine profile of the antigen-presenting cells, influence the adaptive immune response. TLR-2 signals through its TIR (Toll/IL-1 homology region) via MyD88 and the Fas-associated death domain to activate caspase 8 [19]. It also stimulates mycobacterial killing, which is dependent on nitrogen radicals in murine cells, but independent of those in human cells [15]. The mechanism in human cells has yet to be clarified.

ATP stimulates the purinergic receptor P2X7, to promote macrophage apoptosis, but little is known about the signalling pathway involved. P2X7 activation results in calcium influx via an ion channel and, subsequently, by the formation of pores (0.9 kDa) that allow potassium efflux and the entry of large molecules [20]. However, P2X7 is unusual in that it also has a long intracellular C-terminus, which is essential for the induction of macrophage death [21]. This may signal through epithelial membrane protein-2 [21] and, ultimately, the P2X7 apoptosis pathway is known to be caspase 8-dependent [22]. ATP/P2X7 stimulation also causes concomitant mycobacterial killing [23]. The mechanism is independent of nitrogen radicals in both human and murine cells; instead, it is a direct result of mycobacteria-containing phagosomes fusing with lysosomes [24]. Interestingly, ATP/mycobacteria, such as BCG (Mycobacterium bovis Bacille Calmette-Guérin), and the avirulent M. tuberculosis H37Ra, produce more macrophage apoptosis compared with the virulent M. tuberculosis H37Rv [27]. A number of mechanisms by which virulent tuberculosi prevents macrophage apoptosis have been identified, including (1) increased secretion of soluble TNF-α [28], which mops up TNF-α; (2) increased levels of anti-apoptotic proteins, e.g. Mcl-1 [29]; and (3) inactivation of pro-apoptotic proteins, e.g. Bad [30]. Interestingly, increasing macrophage apoptosis by inhibiting Mcl-1 activity leads to a decrease in mycobacterial survival [29]. In vivo inhibition of the adaptive immune response, e.g. down-regulation of dendritic cells by the mycobacterial antigen MannLAM (mannosyl-capped lipoarabinomannan) via their receptor DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) [31], will also cause a reduction in macrophage apoptosis by decreasing the T lymphocyte activity.

**Conclusion**

Macrophage apoptosis occurs within the granuloma. It appears to favour host immunity, but M. tuberculosis is capable of suppressing it partially. Vaccines and immunotherapies that result in increased levels of macrophage apoptosis within the granuloma may be able to tip the balance away from M. tuberculosis virulence and towards a successful host immune response. Alternatively, increased knowledge of the signalling pathways and effector mechanism(s) triggered during apoptosis will lead to new immunotherapies that simulate macrophages to kill M. tuberculosis.

**References**


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