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Review

Innate immunity in tuberculosis: how the sensing of mycobacteria and tissue damage modulates macrophage death

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Abstract

The success of *Mycobacterium tuberculosis* as a human pathogen has been attributed to the ability of the bacillus to proliferate inside macrophages and to induce cell death. This review describes how the sensors of the innate immune system modulate the cell death pathways in infected macrophages and, consequently, the pathogenesis of tuberculosis.

Keywords: Mycobacteria; Innate immune sensors; Danger signal; Inflammatory mediators; Cell death

1. Introduction

Tuberculosis (TB) remains a serious global health problem. Nearly 9 million new annual cases of the disease and 1.5 million deaths per year were reported worldwide in 2013 [1]. Although the numbers of TB cases have progressively declined in recent decades, an increment of 400,000 new cases and 200,000 deaths was reported in the last year. The high incidence of Human Immunodeficiency Virus (HIV) infection and the increase in drug-resistant mycobacteria were implicated to augment TB cases. *Mycobacterium tuberculosis* (Mtb) is typically transmitted by aerosolized droplets and reaches the lungs, where the bacilli are phagocytized by alveolar macrophages, which are the main cell population in the front line of host defense [2]. Inflammatory cells are recruited into the lungs during the early infection and, in most cases, promote non-sterile control of the bacilli. Therefore, mycobacterial infection usually develops as a latent infection in which the equilibrium between the bacillus and the host defense is guaranteed by granuloma formation. The immune response helps to control the proliferation of mycobacteria, but some survive in the intragranulomatous necrotic masses formed as a result of macrophage death. The reactivation of latent TB in immunocompetent individuals occurs at rates that range from 3% to 10% per lifespan [3], and these rates are greatly increased under immunosuppressive conditions caused by HIV co-infection [2,4] or by therapy with anti-Tumor Necrosis Factor (TNF)-α or interleukin (IL)-12/IL-23 blockers [4,5]. In fact, the unbalance of the immune response can compromise the control of Mtb and lead to the development of active TB [5,6].

The hallmark of TB reactivation is the failure of the host immune response to restrain the bacterial growth, inducing new rounds of macrophage necrotic death and an increase in granulomatous lesions, as a result of the recruitment of inflammatory cells [7,8]. In the aggressive cases of pulmonary TB, the exacerbation of necrotic cell death leads to extensive

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caseous lesions and, in consequence, to the rupture of granulomas into airways as well as the cavitation and dissemination of mycobacteria [9]. Massive tissue damage compromises the physiological function of the lungs and culminates in respiratory failure and patient death. In recent years, the molecular mechanisms involved in the death of infected macrophages and the development of necrotic lesions have begun to be elucidated [10–14]. This information is critical for the development of adjunctive therapeutic approaches aimed at decreasing TB-induced necrosis and improving disease outcomes. In this review, we summarize the current knowledge about the signaling pathways of the innate immune system that modulate the death of infected macrophages during TB.

2. Cell death programs involved in the pathogenesis of TB

The death of macrophages following Mtb infection plays a crucial role in the pathogenesis of TB. The cytotoxic activity of mycobacteria in heavily infected macrophages allows their dissemination and, consequently, disease progression. On the other hand, the sensing of the intracellular bacilli by macrophages at the initial stage of infection can trigger host cell death. These cell death processes may anticipate the destruction of infected cells and, therefore, contribute to mycobacterial control. For decades, apoptosis was considered to be the only type of programmed cell death with important functions in the physiology of multicellular organisms. Nevertheless, recent efforts to understand how cells die during biological processes (infectious or not) revealed other host programs of cell death, e.g., pyroptose, pyronecrosis, necroptose and ETosis or NETosis. It has been proposed that macrophage necrosis facilitates the spread of mycobacteria [10,11,15,16], whereas the activation of the apoptotic program contributes to bacillus control [11,14,16]. However, although apoptosis seems to be a good way to eliminate mycobacteria, some studies do not support the apoptotic antimicrobial effect and suggest that this process promotes macrophage deactivation and consequent bacillus growth and dissemination [17,18]. In fact, the particularities of different types of cell death in the outcome of TB are only beginning to be elucidated.

2.1. Apoptotic cell death

Apoptosis is a non-inflammatory type of cell death in which the fragmented nuclear and cytoplasmic contents of dying cells are surrounded by membrane-bound vesicles called apoptotic bodies [19]. The neighboring phagocytic cells engulf the apoptotic bodies by a process called efferocytosis and produce anti-inflammatory cytokines, such as Transforming Growth Factor-β (TGF-β) and IL-10 [20]. Apoptotic cell death is characterized by the activation of caspases-3, -7, -8, -9 and -10; cell shrinkage; chromatic condensation; nuclear fragmentation; plasma membrane phospholipid flip-flop; and plasma membrane blebbing [20–22]. Three distinct molecular pathways can induce the apoptotic process, namely, extrinsic, intrinsic and perforin/granzyme apoptosis. The extrinsic pathway is triggered by ligand binding to TNF receptor (TNFR) family proteins, e.g., TNFR1 (CD120a) and FAS (CD95), which contain a death domain in their cytoplasmic portion that promotes the activation of initiator caspases (8, 9 and 10) and then executioner caspases (3 and 7) [22,23]. The activation of executioner caspases leads to the cleavage of a large spectrum of death substrates that result in DNA fragmentation. In the intrinsic pathway, intracellular stress results in mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c into the cytosol. Cytochrome c binds to the cytosolic protein Apoptotic Protease Activating Factor-1 (Apaf-1) and promotes the subsequent replacement of Apaf-1-associated ADP by deoxy-ATP/ATP, which triggers the formation of a multimeric signaling complex called the apoptosome [24,25]. The recruitment and cleavage of pro-caspase-9 by the apoptosome activates the executioner caspases [21,25]. The B cell lymphoma-2 (Bcl-2) family, a large multigene family that inhibits (e.g., Bcl-2) or induces (e.g., Bcl-2 Interacting Mediator of Cell Death – Bim) apoptosis, regulates the mitochondrial pathway [26]. The release of perforin and granzyme B from the granules of cytotoxic T lymphocytes and natural killer cells triggers the third apoptotic pathway. Perforin creates a pore on the plasma membrane of target cells and allows granzyme B to access the cytosol. Granzyme B cleaves the initiator and executioner caspases [27,28].

Several reports show that Mtb induces apoptotic cell death by extrinsic and intrinsic pathways [16,29,30]. Upregulation of pro-apoptotic genes, such as TNF-α, Fas, Fas ligand, Bim and caspase-3, -5, -7 and -8 [29,31,32], is observed after Mtb infection. Mtb-induced apoptotic gene activation results in elevated levels of DNA fragmentation [15]; capase-3, -8 and -9 activation [29,32,33]; and, consequently, increased annexin V staining in macrophages [34]. Interestingly, virulent mycobacteria are able to modulate the apoptotic cell death through the downregulation of pro-apoptotic genes [30,35].

2.2. Necrotic cell death

Various factors external to cells or tissues, such as infections, toxins or injuries, result in necrotic cell death. Necrosis is an inflammatory type of cell death that is characterized by the swelling of the cell and organelles accompanied by the loss of plasma membrane integrity and release of cytoplasm and nuclear contents [36]. Consequently, a large amount of damage-associated molecular patterns (DAMPs) is released in the extracellular *milieu*. The sensing of DAMPs by structural and resident cells (e.g., endothelial and immunological cells) triggers inflammatory and reparatory responses. Conventionally, necrotic cell death is a passive process that develops without the cellular machinery. However, the discovery of necroptosis showed that cells could execute a necrotic cell death program. This process was originally defined as a backup defense mechanism against viruses that ensures the elimination of infected cells by TNFR1 and TNFR2 signaling in the presence of viral caspase-8 inhibitors [37,38]. The immunogenicity of necroptosis helps to target pathogens by the immune system. This process