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REVIEW

Neutrophil apoptosis in the context of tuberculosis infection

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SUMMARY

Polymorphonuclear neutrophils comprise two-thirds of peripheral blood leukocytes and are key components of innate immunity as a first line of defense against bacterial and fungal pathogens. Their microbicidal mechanisms are essential for bacterial killing, the enhancement of inflammatory reactions and also comprise signaling molecules which have been implicated in signal transduction cascades. In tuberculosis, the number of neutrophils increases in the affected lung. In addition, they become activated and apoptotic due the bacterial burden. As apoptosis is promoted by reactive oxygen species (ROS) during phagocytosis, the advantages and benefits to the host as well as the strategies displayed by the pathogen to avoid or retard apoptosis are discussed in this review.

1. Neutrophils and apoptosis

The influx of neutrophils to the lung is one of the first events in the pathogenesis of tuberculosis [1] playing a main role in the acute phase of the disease [2]. Neutrophils display microbicidal mechanisms that include the release of proteolytic enzymes, antimicrobial peptides, and the rapid production of ROS [3]. Since the excessive neutrophil activation might cause severe tissue damage and inflammatory diseases [4], certain mechanisms have been evolved in order to resolve inflammation. Programmed cell death in neutrophils, or apoptosis, constrains the release of inflammatory mediators and toxic granule constituents [5] through the recognition and phagocytosis of the bacilli by macrophages, a process that is essential to resolve inflammation [6]. In addition, it has recently been reported a new-ROS dependent cell death mechanism, which leads to neutrophil extracellular traps (NETs) and induces NET-mediated killing [7].

2. Neutrophil apoptosis and tuberculosis

As the disease evolves, the number of neutrophils increases in the bronchoalveolar lavage fluid of the affected lung [8]. Although this mobilization is important for microbicidal function, the powerful mediators such as oxidants and elastase which help neutrophils to be effective killers, can also injure endothelial cells and produce structural damage [9]. In line with this, it has been demonstrated that neutrophils from patients with active tuberculosis display an increased spontaneous apoptosis in vitro [10,11] which is related to the activation state of these cells in bloodstream [12]. Pathogen-induced cell death often involves the modulation of apoptosis and, accordingly, the activation of neutrophils induced by *Mycobacterium tuberculosis (Mtb)* accelerates neutrophil apoptosis in vitro [12–14].

Cytokines and cytokine-producing cells are present in pleural effusions from people with malignant and infectious diseases [15]. Patients with tuberculous pleuritis have elevated amounts of tumor necrosis factor (TNF)-α [16,17], transforming growth factor-β [17,18], interferon-γ [11,16,19], interleukin (IL)-8 [20], IL-6 [21], IL-18 [19], and IL-10 [17], which may modulate the lifetime of neutrophils. In this context it has been described that, after extravasation at the infected lung, the expression of those receptors associated with activation/apoptotis (CD11b, CD64, TNF-R55, and FasL) is up-regulated in neutrophils [22]. Therefore, the cytokine milieu in the pleural space appears to influence the signaling pathways on activated neutrophils, leading to apoptosis and inhibiting proinflammatory capacity.

A large body of evidence has now indicated that neutrophils participate as a bridge between innate and adaptive immune

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responses, for example by acquiring DC markers [22,23], or by leading to specific T lymphocyte responses by crosspresentation [24]. In this regard, phagocytosis of apoptotic bodies promotes a more efficient fusion of the phagosome with the lysosome which results in the digestion of the pathogen and promoting antigen presentation to T cells [25]. Therefore, we can consider the inflammatory milieu as a place subjected to a delicate balance between the infected and uninfected neutrophils, limiting inflammation and/or the generation of specific immune activity.

3. Neutrophils and Toll-like receptors

The ability to discriminate between self and non-self is a process in which pattern-recognition receptors such as the Toll like receptors (TLRs) respond to evolutionary conserved non-self proteins [26]. TLRs are members of the IL-1R superfamily whose signaling pathways involve mitogen-activated protein kinase (MAPK) cascades, including c-Jun N-terminal kinase, p38, and extracellular signal-regulated kinases (ERK) MAPK. NF-κB is a heterodimer composed of p50 and p65 subunits which, in resting cells, remains in the cytoplasm because of its interaction with inhibitory κB (IκB) proteins. However, the phosphorylation events which follow TLR stimulation, lead to polyubiquitination and degradation of IκB, allowing nuclear translocation of NF-κB subunits to bind DNA and to trigger transcription of pro-inflammatory cytokine genes [27]. Moreover, the response of neutrophils to cytokines [28] and their activation [29] also involves p38 and ERK MAPK pathway. Human neutrophils express an array of TLRs like TLRs 1, 2, 4, 5, 6, 7, 8, 9, and 10 [30] involved in interleukin-8 (IL-8) secretion, an essential event in neutrophil migration to the lung [31].

4. *Mtb* and TLR signaling in neutrophils

Recognition of mycobacterial components by TLRs is a key step in initiating innate immune responses upon mycobacterial infection. In particular, TLR2 appears to be critical for sensing mycobacteria inducing a crucial proinflammatory signal, TNF-α [32].

Signaling pathways triggered by TLRs have been previously implicated in the regulation of neutrophil lifespan [33,34] involving tyrosine phosphorylation events [35]. In fact, it has been shown that *Mtb* activates p38 MAPK in neutrophils in vitro via the TLRs pathway inducing its activation and apoptosis [14,36]. These findings are coherent with high expression of the activated form of this kinase in peripheral blood as well as in pleural effusion neutrophils from tuberculosis patients [22]. Pathogens unable to bind directly to TLRs likely activate signaling complexes via lipid raft reorganization involving numerous cell surface proteins [37]. In this context, a variety of pathogens interact with lipid rafts which are involved in the attachment, cell entry, and intracellular survival of several microorganisms such as *Brucella suis* [38], *Chlamydia trachomatis* [39], and *Mycobacterium bovis* [40]. In addition, membrane lipid rafts are signaling platforms enriched in cholesterol that are required for innate immune responses [41] mediating the efficiency of oxidase coupling to receptors in the neutrophil membrane. On the other hand, TLR2 cooperates with other receptors in lipid rafts on macrophages and DC membrane, for the production of inflammatory cytokines in response to fungal wall-derived β-glucans [42]. Furthermore, it has also been reported that TLR2/dectin-1 cooperation induces ROS production, together with the rapid and sustained phosphorylation of p38 and Syk in monocytes [43,44], and neutrophils [45,46] in response to mycobacteria.

5. Neutrophils and reactive oxygen species

Phagocytes produce ROS through activation of nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase that is assembled at the plasma membrane. Although the ability of ROS to kill *Mtb* has been demonstrated only in mice [47], the increase in superoxide and hydrogen peroxide could affect important signaling enzymes necessary for physiologic cell signaling. For instance, ROS participate in TLR2 signaling pathways together with a sustained phosphorylation of p38 suggesting an essential role of ROS in TLR2 signaling pathways [48]. In addition, neutrophil apoptosis is dependent on the generation of ROS during the phagocytosis of *Mtb* [13,14] involving the activation of p38. Therefore, *Mtb*-induced respiratory burst in neutrophils can also regulate the inflammatory response by induction of apoptosis.

It has been demonstrated that ROS activate NF-κB by an indirect mechanism [49] through the oxidation of thioredoxin (Trx) that in turn release the transcription factor complex protein-1, which is an upstream kinase in the c-JunN-terminal and p38 kinases [50], permitting its activation and downstream signaling [51]. Human neutrophils express several caspases, cysteine-dependent enzymes and thus potentially redox sensitive, which are key enzymes in apoptosis [52]. In this context, it has been described that *Mtb*-induced apoptosis in neutrophils is dependent on caspase-3, which is dependent on ROS and it has also been shown to be rapidly activated by *Mtb* [14].

6. Neutrophils and *Mtb* immune evasion

The ability of *Mtb* to infect and cause disease depends on its capacity to evade killing by phagocytic cells and to achieve this, *Mtb* is equipped with numerous strategies of immune evasion. For instance, it has been reported that virulent mycobacteria are able to escape from fused phagosomes and multiply [53]. In addition, *Mtb*-infected macrophages appear to be diminished in their ability to present antigens to T cells, which leads to persistent infection [54] and even more, *Mtb* has developed mechanisms to prevent both DC migration and antigen presentation [55,56]. In this way, antigen presenting cells contribute to defective T cell proliferation and function by the production of cytokines including TGF-β, IL-10 [57] or IL-6 [58]. In addition, neutrophils impair *Mtb*-induced DC maturation, thereby limiting their capacity to stimulate T cells [24].

Several pathogens could avoid neutrophil killing by limiting phagocytosis [59,60] and/or decreasing ROS production [61–64]. In line with this, *Mtb* could employ CD11b for entry, which do not induce respiratory burst [65]. Additionally, inhibition of ROS production also delays apoptosis in neutrophils and therefore, anti-apoptotic properties of a pathogen could be considered as a mechanism of immune evasion in which neutrophil survival is controlled by the bacilli allowing its persistence inside the host cell. This is coherent with the fact that attenuated strains of *Mtb* have been found to be much more effective than virulent strains at inducing apoptosis [66]. In this manner, it has been recently described that some *Mtb* strains fail to induce apoptosis due to an inability to stimulate ROS production and the induction of anti-apoptotic pathways [13]. This strategy has been also described for other pathogens like *Anaplasma phagocytophilum* which fails to trigger ROS and delays neutrophil apoptosis [68].

Other pathogen abilities rely on their capacity to secrete superoxide dismutase and catalase which are ROS antagonists [68], as well as sulphatides, LAM and phenolic-glycolipid I (PGLI) which are potent oxygen radical scavengers [69]. In terms of host genetics, it has been proposed that there is a direct association between of gene polymorphisms in patients with tuberculosis (i.e.

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