REVIEW ARTICLE

Cytokines modulate neutrophil death

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ABSTRACT. Polymorphonuclear neutrophils (PMN) are terminally differentiated cells with a short life span, in the blood circulation. The prolongation of the life span of PMN is critical in their effectiveness against pathogens, in particular in the tissues. This review summarizes the effect of cytokines on PMN apoptosis and points to some examples of pathological situations characterized by inappropriate cytokine production associated with dysregulation of PMN apoptosis.

Keywords: neutrophils, apoptosis, cytokines, inflammation

Polymorphonuclear neutrophils (PMN) are key components of the first line of defense against bacterial and fungal pathogens. They contribute to the early, innate response by rapidly migrating to inflamed tissues, where their activation triggers microbicidal mechanisms such as the release of proteolytic enzymes and antimicrobial peptides, as well as the rapid production of reactive oxygen species (ROS) in what is called the oxidative burst. ROS are essential for bacterial killing and also potentiate inflammatory reactions [1].

PMN are usually short-lived cells, which die spontaneously by necrosis or apoptosis. Apoptotic PMN are recognized and phagocytosed by macrophages, a process that is essential to resolve inflammation [2]. In fact, this phagocytic removal of intact, apoptotic neutrophils prevents them from releasing their cytotoxic content into the extracellular environment, which would occur if the cells died by necrosis [3]. The prolongation of PMN life span is critical in their effectiveness against pathogens. Shortened PMN survival due to apoptosis may contribute to susceptibility to severe and recurrent infections, in some pathological situations, through neutropenia [4, 5]; in addition, down-regulation of the pro-inflammatory capacity of PMN has been reported during apoptosis [6]. In contrast, inappropriate PMN survival and persistence at sites of inflammation are thought to contribute to the pathology of chronic inflammatory diseases [7, 8]. Thus, programmed death in PMN needs to be well regulated in order to provide an appropriate balance between their immune functions and their safe clearance.

In this context, it has been shown that cytokines have a crucial role in determining PMN cell survival. This review gives an overview of the cell signalling involved in cytokine modulation of PMN death.

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MOLECULAR MECHANISMS OF NEUTROPHIL APOPTOSIS *(FIGURE 1)*

Role of caspases

PMN apoptosis involves the activation of a family of cysteine proteases, called caspases, which cleave cellular substrates at an obligatory aspartic acid within a preferred sequence [9]. Caspase activation is a central event in apoptosis, and results in the proteolytic degradation of multiple substrate proteins that contribute to the apoptotic phenotype. PMN express a variety of regulatory and effector caspases, including caspases-1, -3 and -8 [10, 11]. PMN contain barely detectable levels of cytochrome *c*; however, the trace amount of cytochrome *c* present in PMN is both necessary and sufficient for caspase activation [12]. More recently, it has been proposed that cathepsin D, a serine protease localized in the azurophilic granules, mediates caspase-8 activity [13].

Role of calpains

Calpains are also cysteine proteases present in isolated PMN [14]. The level of calpastatin, a highly specific calpain inhibitor, decreases during PMN death, leading to a drastic enhancement of the calpain-1 activity. Activated calpain-1 cleaves, in turn, the proapoptotic molecule Bax into an active fragment [15]. Furthermore, it has been reported that calpain mediates the cleavage of Atg5, an autophagy-related gene required for the formation of autophagosomes, switching autophagy to apoptosis [16].

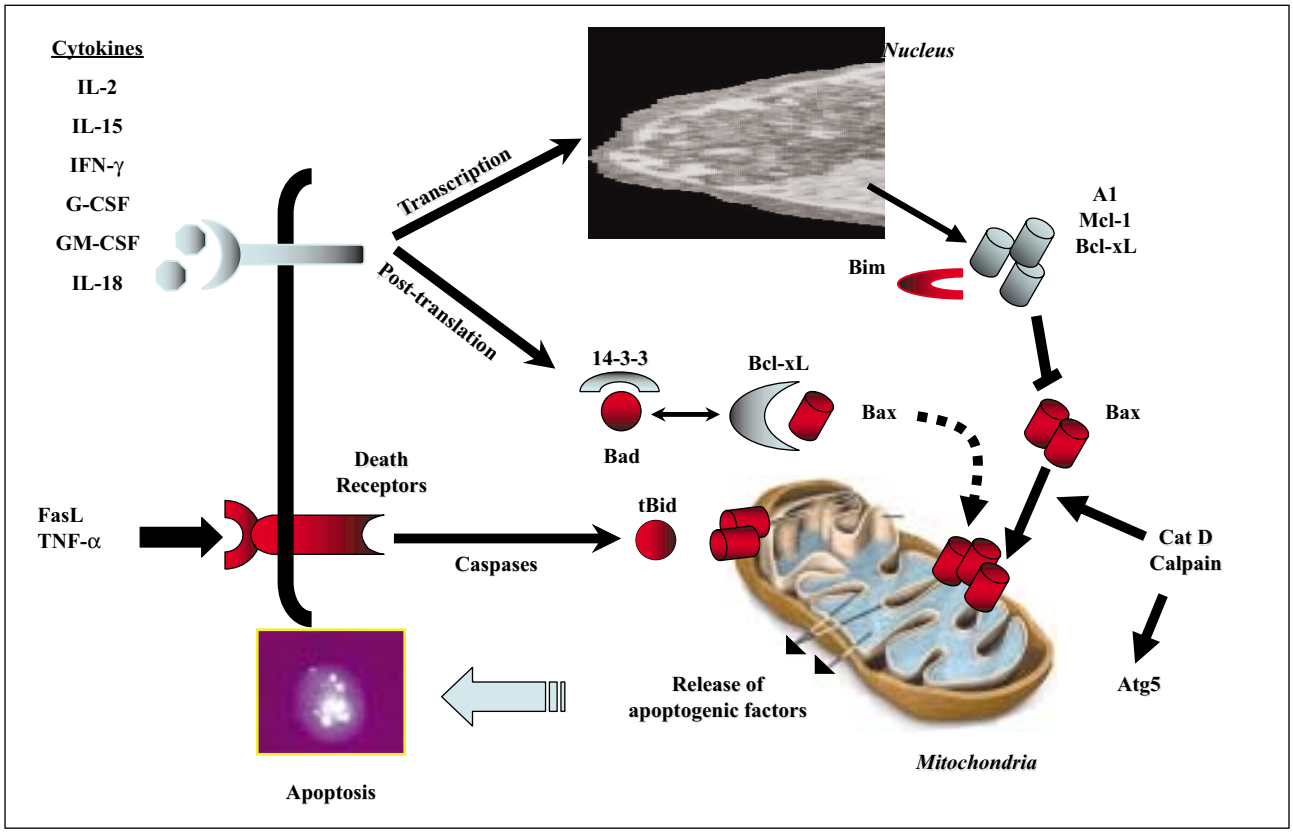

Figure 1

Biochemical pathways involved in the death of neutrophils.

Members of the Bcl-2 family

It is now generally agreed that PMN do not express the anti-apoptotic protein Bcl-2, but they do express mRNA for the anti-apoptotic proteins, Mcl-1, A1 and Bcl-xL [17, 18]. Mcl-1 and A1 proteins are expressed in PMN, and their levels decrease prior to the onset of apoptosis [17, 18]. Mcl-1 and A1 proteins have very short half-lives (approximately 2-3 h), whereas the half-lives of the pro-apoptotic proteins such as Bax, Bak and Bad, are relatively long. In the absence of *de novo* synthesis of Mcl-1 and A1, the activity of the longer-lived pro-apoptotic proteins prevails and tips the balance towards apoptosis.

Members of the TNF family

The TNFR is a transmembrane protein containing an extracellular TNF-binding domain and a TNFR-associated death domain (TRADD) in the cytoplasmic region of the protein [19]. PMN express two TNFRs, TNFRSF1A (55-R, CD120a, or TNFRI) and TNFSFR1B (75-R, CD120b, or TNFRII), and each has a slightly different role in PMN apoptosis [20]. Gon *et al.* showed that TNFRI is required for TNF-α-mediated PMN apoptosis, and its ability to promote apoptosis is enhanced by TNFRII [21]. Blocking TNFRI, but not TNFRII, with specific antibodies inhibits neutrophil apoptosis [21]. Additional work using TNFR-selective mutants, has shown that TNFRI is dominant [22]. Moreover, the ability of TNF-α to induce PMN apoptosis was reported to be linked to ROS production. Indeed, PMN from patients with chronic granulomatous disease, characterized by a defect in ROS production, fail to undergo apoptosis in the presence of high concentrations of TNF-α [20, 23].

IN VITRO MODULATION OF PMN DEATH BY CYTOKINES *(TABLE 1)*

Among the various pro-inflammatory cytokines, it has been shown that *in vitro* IL-1β, IL-2, TNF-α, IL-15, IFN-γ, G-CSF, GM-CSF and IL-18 can prolong PMN survival [24, 25]. IL-8, a chemokine, has also been shown to delay PMN apoptosis mediated by Fas and TNF-α receptors [26, 27]. The reported effects of IL-6 on PMN apoptosis are however, more controversial [28, 29]. In this context, G-CSF and GM-CSF exert potent *in vitro* stimulatory effects on PMN from HIV-infected patients at the late stage of the disease [30, 31]. Similarly, IL-15 significantly enhanced *in vitro* PMN functional activity and decreased PMN cell death in PMN from untreated advanced HIV-infected patients [32].

Notably, TNF-α has been shown to have both pro-apoptotic and anti-apoptotic effects toward PMN. Van de Berg *et al.* showed that this bipolar effect is concentration-dependent [20]. At low concentrations (> 0.1 ng/mL), TNF-α delays PMN apoptosis and elicits production of proinflammatory cytokines, whereas at higher concentrations TNF-α initiates apoptosis. Consistent with these observations, high concentrations of TNF-α (10-100 ng/mL) override the ability of IFN-γ and GM-CSF to delay apoptosis [20].

Table 1
Inhibition of PMN apoptosis by cytokines

Cytokines able to inhibit PMN apoptosis	- IL-1β, IL-2, TNF-α, IL-15, IFN-γ, IL-8, IL-18, G-CSF, GM-CSF [28-31]
Mechanisms	1) Effect on anti-apoptotic molecules - Increased expression of Mcl-1 [17, 33-35] - Increased expression of A1 [41] 2) Effect on pro-apoptotic molecules - Decreased expression of Bax [42, 43] - Increased phosphorylation of Bax or Bad [36, 44] leading to decreased pro-apoptotic activity of Bax 3) Post-mitochondrial control - Inhibition of calpain activity [48]
Pathological situations associated with delayed PMN apoptosis and increased levels of pro-inflammatory cytokines	- Acute respiratory distress syndrome [50, 23] - Sepsis [51], acute pneumonia [42] - Rheumatoid arthritis [52] - Inflammatory bowel disease [54] - Cystic fibrosis; idiopathic fibrosis [55, 56] - Unstable angina and acute myocardial infarction [57] - Cancer associated with neutrophilia [42]
Therapeutic administration of G-CSF and GM-CSF in pathological situations associated with increased PMN apoptosis	- Community acquired pneumonia [58] - Cancer [59] - Cyclic neutropenia [60]

Enhanced expression of the anti-apoptotic protein Mcl-1 has been implicated in GM-CSF and IL-15-delayed PMN apoptosis [17, 33-35]. While transcriptional up-regulation of Mcl-1 is correlated with MAPK/Erk1/2 kinase activation [36], increased translation of Mcl-1 has been shown to depend on the PI-3K/Akt pathway [37]. Lyn kinase, Janus kinase/signal transducer and activator of transcription, and CD137 have also been reported to play prominent roles in GM-CSF-mediated survival through increased-Mcl-1 expression [38-40]. Early increases in Mcl-1 expression may represent phosphorylation or stabilization of Mcl-1 protein. Upregulation of the anti-apoptotic protein A1 has also been shown to be involved in GM-CSF-induced PMN survival [41].

Conversely, a decreased expression of the pro-apoptotic protein Bax has been observed in aged PMN stimulated with G-CSF, GM-CSF, IL-6 and IL-15, suggesting that the anti-apoptotic effect of these cytokines is, in part, related to the inhibition of Bax [42, 43]. Increased phosphorylation of the pro-apoptotic molecule Bad has been shown to be involved in PMN survival induced by GM-CSF [36, 44]. Phosphorylation results in the binding of Bad to the cytoplasmic 14-3-3 protein that interrupts the association between Bad and Bcl-X_L. Increased amounts of Bcl-X_L are then free to bind with Bax and prevent its proapoptotic activity. Finally, increased Bax phosphorylation has also been reported to regulate its activity, leading to increased PMN survival following GM-CSF and G-CSF-treatment [45]. The phosphorylation of Bad and Bax required PI3K/Akt activation and appeared to be mediated by Akt itself [44-46]. Moreover, death by neglect of PMN involves upregulation of the pro-apoptotic BH3-only member named Bim that is counteracted by GM-CSF [47]. Finally, G-CSF has also been recently reported to inhibit PMN apoptosis by inhibition of post-mitochondrial calpain activity upstream of caspase-3 [48]. Interestingly, Lichtner *et al.* have reported that HIV protease inhibitors reverse *in vitro* apoptosis of PMN from AIDS patients by inhibiting calpain activity [49].

IN VIVO PMN DEATH AND IMMUNO-MODULATING EFFECT OF CYTOKINES *(TABLE 1)*

The lifespan of PMN increases significantly once they migrate out of the circulation and into the sites of inflammation, where they encounter various pro-inflammatory mediators. It has been extensively demonstrated that delayed PMN apoptosis is associated with increased pro-inflammatory cytokine levels in several diseases such as acute respiratory distress syndrome (ARDS) [50], sepsis [51], rheumatoid arthritis [52], cystic fibrosis, idiopathic fibrosis, acute pneumonia, and cancer associated with neutrophilia [42]. In particular, dramatically elevated levels of IL-2 have been observed in lung fluids of patients with early ARDS. IL-2 associated with GM-CSF and G-CSF significantly contributes to the inhibition of PMN apoptosis in bronchoalveolar lavage fluids of patients with ARDS [53]. Increased mucosal production of G-CSF is also related to a delay in PMN apoptosis in inflammatory bowel disease (IBD) [54], thus providing a possible mechanism for tissue accumulation of PMN in IBD. Enhanced PMN survival in airways has been reported in patients with cystic fibrosis and has been related to increased expression of G-CSF and GM-CSF [55]. Garlichs *et al.* [56] reported a pronounced delay of PMN apoptosis in patients with unstable angina and acute myocardial infarction (ACS) associated with increased serum levels of IFN-γ, GM-CSF, and IL-1β. Serum from ACS patients inhibits apoptosis of PMN from healthy controls. Inflammatory cytokines (IL-6, IL-8) during cardiopulmonary bypass prolong the functional lifespan of PMN through modulation of apoptosis and potentiate the inflammatory response observed after coronary bypass operation [57]. Thus, the ability of various proinflammatory molecules to delay PMN apoptosis is likely to be important in the initiation of pathological inflammatory responses.

Based on these observations, hematopoietic growth factors, especially G-CSF and GM-CSF, have been found to be effective in various pathological situations associated with neutropenia related to increased apoptosis. In particular, the increased PMN apoptosis reported in patients with community-acquired pneumonia is reversed by G-CSF treatment; prolonged PMN survival is associated with a sustained release of anti-inflammatory cytokines [58]. Similarly, PMN from children with cancer that have defective functional activity and accelerated apoptosis are corrected by G-CSF and GM-CSF *in vitro* [59]. Cyclic neutropenia, due to a mutation in the gene for neutrophil elastase (ELA2), is also effectively treated with G-CSF [60].

During the last decade, the use of non-human primate models has allowed investigation the events involved in SIV infection in terms of virus dynamics and immune responses [61-65]. We recently reported that PMN from

SIV-infected Rhesus macaques (RM), chronically infected with the virulent strain SIVmac251, display increased susceptibility to undergo apoptosis [66]. PMN apoptosis was significantly increased in RMs progressing faster to AIDS as compared to non-progressors RMs. PMN death was also occurring early after infection and was prevented by inhibition of calpain activation but not caspase activation [67]. Interestingly, levels of inflammatory cytokines IL-8 and IL-1β that prevent *in vitro* PMN death, were lower during the chronic phase in RMs progressing towards AIDS. Thus, this decrease in inflammatory cytokines might lead to an abnormal tendency of PMN to die. However, further studies are necessary to evaluate the *in vivo* effect of anti-apoptotic cytokines in non-human primate models as a preclinical phase for HIV-infected individuals.

Finally, individuals with TNFR-associated periodic syndrome (TRAPS) have a defect in the TNFR and, therefore, diminished PMN apoptosis [68]. Patients with TRAPS experience recurrent attacks of fever lasting > 1 week that is associated with abdominal pain, severe arthromyalgias, rash, and periorbital edema. However, TRAPS has not been associated with increased infections [68].

CONCLUSION

Because PMN are the most abundant leucocytes in the circulation, and as they provide a primary, innate immune defense against a wide range of microbial infections before the development of a specific immune response, understanding the mechanisms that control their exhaustion in the bone marrow, trafficking and survival may have potential benefits for human diseases.

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