Role of Neutrophils Apoptosis in Osteomyelitis Pathogenesis

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Editorial

Osteomyelitis is a bone infection characterized by progressive inflammatory destruction of the infected bone and new apposition of bone at the site of infection. In adults, osteomyelitis is usually a complication of open wounds due to fractures, surgery, or both, with or without the presence of foreign bodies such as prosthetic devices. In fact, it is estimated that about 0.4 to 7% of trauma and orthopaedic interventions are complicated by osteomyelitis [1-6].

Bone infections can also be the result of bacteraemia, mostly in children and in elderly patients, in whom the infection involves mainly the axial skeleton. The microorganism most frequently isolated in both post-traumatic and haematogenous cases is *Staphylococcus aureus*. Osteomyelitis constitutes a difficult-to-treat infection, with high rates of recurrence of about 20-30% despite appropriate medical and surgical therapies, causing significant morbidity and mortality [7-13].

Much attention has been dedicated to improving the medical and surgical treatments of osteomyelitis, but little progress has been made toward understanding its pathogenesis. It is clear that it is multifactorial and influenced mainly by local factors related to the bone lesion and microorganisms inoculated into the bone, but inherited factors and cell immunity dysfunctions could play some role as well [14-26].

The neutrophils in the pathogenesis of osteomyelitis

Neutrophils are key elements in the cellular defense against bacterial infections. Therefore, changes in their apoptotic regulation in patients with osteomyelitis might modify their phagocytic activity or lifespan, leading to suboptimal responses to infection.

Neutrophils play a dual activity in the focus of infection. On one hand they contribute to phagocytosis and elimination of bacteria. On the other hand phagocytosis of bacteria by activation of neutrophils elicits production of NADPH oxidase-derived superoxide, which is converted into other highly toxic reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play a critical role in the bacteria-induced apoptosis and/or in the phagocytosis-induced cell death [27-29]. Neutrophils lysis also promotes host tissue destruction and inflammation. In addition to the neutrophils, there is also a mobilization of macrophages that work as true scavengers, removing apoptotic neutrophils and contributing to tissue repair (Figure 1).

In summary, the delay of neutrophils' apoptosis at the infection focus would contribute to the resolution of the infection, while its acceleration would favour the persistence of the infection. Therefore, it is critical to understand the mechanisms of apoptosis of the neutrophils and its regulation in osteomyelitis and other bacterial infections.
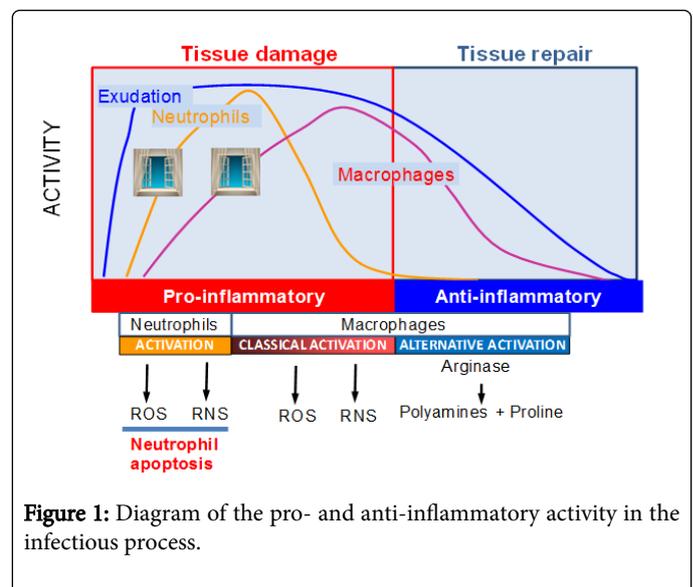

Figure 1: Diagram of the pro- and anti-inflammatory activity in the infectious process.
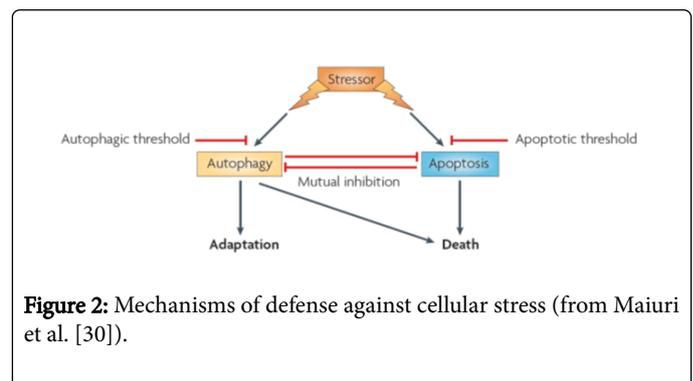

Figure 2: Mechanisms of defense against cellular stress (from Maiuri et al. [30]).

### Mechanisms of defense against a stress or cellular aggression

Autophagy is a natural and self-destructive process in which the cell eliminates unnecessary or non-functional elements. The cell might respond against a stressor or nocive agent by activating autophagic and/or apoptotic mechanisms, leading to an adaptation to the stress or to cell death. The intensity of the stress with different sensitivity

thresholds will determine if the cell develops autophagy or apoptosis. The choice between apoptosis or autophagy is also influenced by the fact that the two catabolic processes show some degree of mutual inhibition. A mixed phenotype of apoptosis and autophagy can be detected at the single-cell level. Although autophagy mostly allows the cell adaptation to stress, massive autophagy can also kill cells as apoptosis does (Figure 2) [30].

Differences between apoptosis and necrosis

There is a clear difference between apoptosis (programed cell death) and necrosis (accidental death). During necrosis there are changes in the cellular nucleus with chromatin clumping, and swelling of the organelles, including flocculent mitochondria. The overall process leads to disintegration of the cell and release of its intracellular contents producing inflammation. However, during apoptosis there is shrinkage of the cellular nucleus with chromatin compaction and segregation, and cytoplasm condensation. In a further step, nuclear fragmentation, blebbing and formation of apoptotic bodies occur. As opposed to necrosis, there is no inflammation in apoptosis (Figure 3).
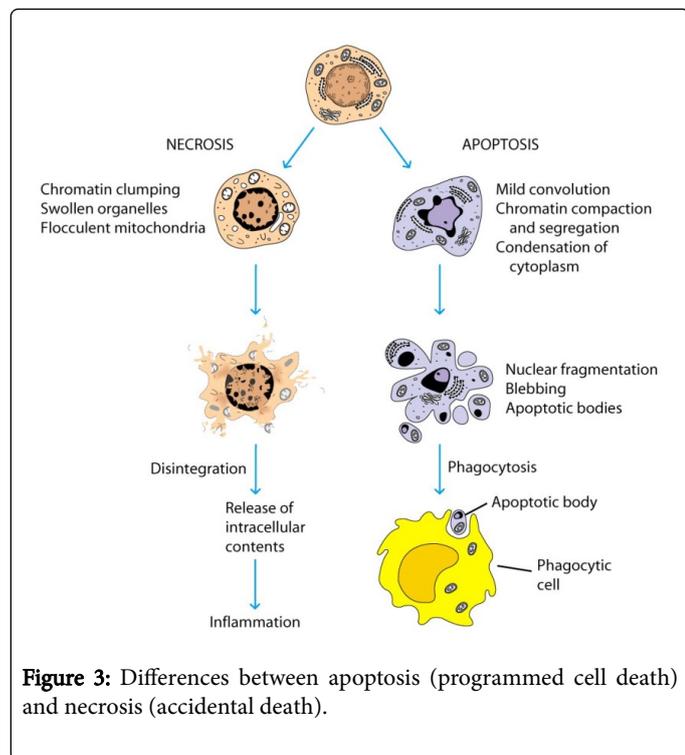

Figure 3: Differences between apoptosis (programmed cell death) and necrosis (accidental death).

Cellular apoptosis induction and regulation pathways

Cellular apoptosis has two triggering pathways (Figure 4). The extrinsic one is mediated by receptor-ligand interactions through Fas and TNF receptors. The other is an intrinsic pathway, in which sensors of the Bcl-2 family detect some deficiency of growth factors, survival signals, DNA damage or protein misfolding.

Once these receptors are activated, apoptosis starts at the mitochondrial level, where different stimuli block anti-apoptotic proteins (Bcl-2, Bcl-xl) and activate pro-apoptotic proteins (Bax, Bak) [31-34]. Pro-apoptotic proteins initiate the caspases pathway leading to DNA and nuclear fragmentation, breakdown of the cytoskeleton and formation of apoptotic bodies that are phagocyted by macrophages.

Other changes observed in apoptosis include modifications in the cell membrane with phosphatidylserine externalization, pH acidification and increase of Ca^{2+} concentration at the intracellular level.
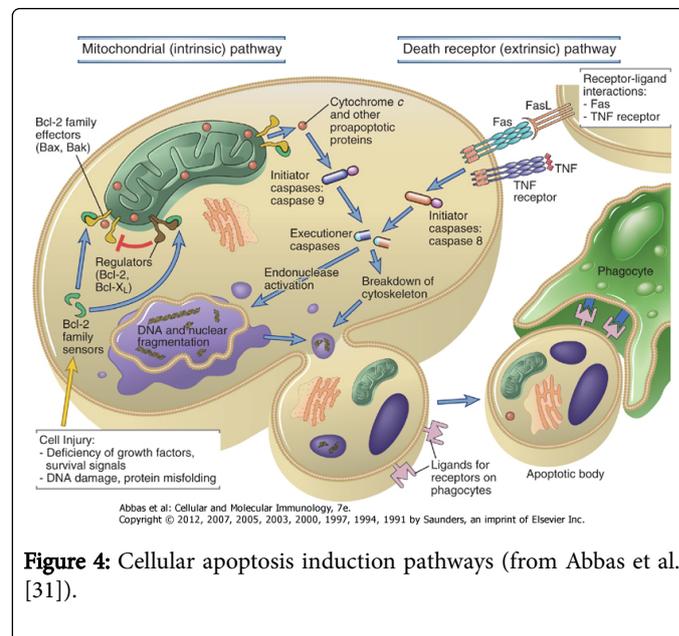
Figure 4: Cellular apoptosis induction pathways (from Abbas et al. [31]).

It has recently been reported that stimulated neutrophils generate a burst of ROS that induce DNA damage signaling in activated neutrophils and macrophages, suppressing cytokine production and inducing apoptosis. If the DNA damage reparative systems are effective cells will survive, otherwise they will become apoptotic and die [35-37].

Delay of neutrophils apoptosis in osteomyelitis

Neutrophils of peripheral blood of patients with osteomyelitis showed a delayed apoptosis compared to neutrophils from healthy donors, an anti-apoptotic effect that seems to be serum-mediated [38]. Patients with osteomyelitis have increased serum levels of C-reactive protein and several cytokines, especially IL-6, a cytokine usually associated with infection [38]. An inverse correlation between serum levels of IL-6 and neutrophils apoptosis has been observed, and the anti-apoptotic effect of osteomyelitis serum reverted after its incubation with an anti-IL-6 monoclonal antibody [38]. These observations support that the anti-apoptotic effect of osteomyelitis serum is related to IL-6.

Delay of neutrophils apoptosis after bacterial phagocytosis

Bacterial phagocytosis influences the "*in vitro*" apoptosis of neutrophils, an effect that seems to be dependent of the bacteria/ neutrophil ratio. Thus, for a bacteria/neutrophil ratio of 1/1, phagocytosis of *S. aureus* and *E. coli* delayed neutrophils apoptosis as compared to controls, whereas for higher bacteria/neutrophil ratios (10/1 to 100/1) bacterial phagocytosis increased neutrophils apoptosis. The anti-apoptotic effect was observed as well after incubation of neutrophils with LPS or lipoteichoic acid, components of the cellular wall of Gram negative and Gram positive bacteria, respectively [39].

Also, phagocytosis of *S. aureus*, and *E. coli* and incubation with LPS and lipoteichoic acid increased the "*in vitro*" production of IL-6, IL-1β and TNF-α by the neutrophils, and the incubation with anti-IL-6 and

anti-TNF-α monoclonal antibodies reduced the anti-apoptotic effect of bacterial phagocytosis at low bacteria/neutrophil ratios [36].

Regarding the pro- and anti-apoptotic proteins regulation, bacterial phagocytosis at low bacteria/neutrophil ratios delayed the apoptosis of the neutrophils by decreasing the expression of Bax-α pro-apoptotic protein and the Bax-α/Bcl-Xl proteins ratio, and the incubation with recombinant de IL-6 delayed the apoptosis of the neutrophils by decreasing the Bax-α/Bcl-Xl proteins ratio [39].

Genetic aspects and apoptosis of the neutrophils

Different reports have shown that the *G(-248)A* polymorphism of the promoter of the *BAX* gene delayed cellular apoptosis of leukemic cells [40,41], and this polymorphism could also influence the lifespan of peripheral blood neutrophils of patients with osteomyelitis [42].

In fact, carriers of the *A* allele of the *G(-248)A* polymorphism of the promoter region of the *BAX* gene were more frequent among osteomyelitis patients compared to healthy donors, and the apoptosis of neutrophils of carriers of this variant *A* allele was delayed compared to carriers of the wild type *GG* genotype of this polymorphism [42].
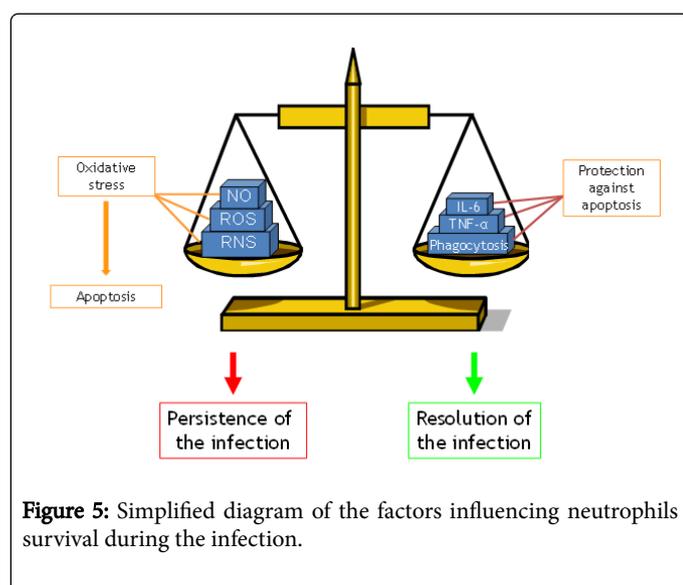
Figure 5: Simplified diagram of the factors influencing neutrophils survival during the infection.

Effect of neutrophils apoptosis on the outcome of the infection

There are factors associated with stress such as nitric oxide (NO) and the formation of ROS and RNS that accelerate the apoptosis of the neutrophils and contribute to perpetuate the infection. On the other hand, bacterial phagocytosis at low bacteria/neutrophil ratios (1/1) and cytokines IL-6 and TNF-α delay the apoptosis of the neutrophils and would contribute to resolve the infection (Figure 5).

Conclusions

Neutrophils are the first line of the host immune defense against microorganisms, especially bacteria. Osteomyelitis patients have a delayed apoptosis of peripheral blood neutrophils. This apoptotic delay seems to be due to the autocrine production of IL-6, induced by bacterial phagocytosis, which decreases the Bax-α/Bcl-Xl proteins expression ratio. This increased lifespan would allow the neutrophils to remain longer in the septic focus and might help to resolve the

bacterial infection. Genetic aspects, such as the carriage of the *G(-248)A* polymorphism of the promoter of the *BAX* gene, seems also to delay neutrophils apoptosis and, consequently, could favour the resolution of the infection. Additional studies are necessary to evaluate the role of apoptosis in osteomyelitis and to clarify if patients with delayed neutrophils apoptosis have better osteomyelitis outcomes in terms of cure and sequelae than those with normal or reduced neutrophils lifespan.

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