New insights into the mechanisms controlling neutrophil survival

Mercedes Cabrini, Karen Nahmod and Jorge Geffner

Department of Immunology, IIHEMA, National Academy of Medicine, Buenos Aires, Argentina

Correspondence to Dr Jorge Geffner, PhD, Department of Immunology, IIHEMA, National Academy of Medicine, Pacheco de Melo 3081 (1425), Buenos Aires, Argentina
Tel: +54 114 805 5696; fax: +54 114 803 9475; e-mail: geffnerj@fibertel.com.ar

Current Opinion in Hematology 2010, 17:31–35

Purpose of review

Neutrophil survival is regulated by a complex convergence of different pathways. The present review analyzes these pathways and discusses how neutrophil survival is modulated during the course of inflammatory reactions.

Recent findings

Although apoptosis appears to be the predominant cell death pathway in the neutrophil, recent data reveal that neutrophil survival is also regulated by a number of nonconventional pathways including NETosis, autophagic cell death, and other less characterized mechanisms. This supports an even more complex picture of the mechanisms involved in the regulation of neutrophil survival than previously thought.

Summary

The control of neutrophil survival is central to homoeostasis and resolution of inflammation. Cell death is usually discussed dichotomously in terms of apoptosis or necrosis. There are two main pathways responsible for the stimulation of apoptosis; a death receptor pathway triggered by Fas, tumor necrosis factor α, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and a mitochondrial pathway stimulated by a number of stressors such as DNA damage, growth factor deprivation, and chemotherapy drugs. Nonconventional pathways of neutrophil death include NETosis and autophagic cell death as well as a number of poorly characterized mechanisms. Understanding the integrated pathways responsible for the control of neutrophil survival holds therapeutic promise in infectious and inflammatory diseases.

Keywords

apoptosis, caspases, extracellular traps, NETosis, neutrophils, oxygen-reactive species

Curr Opin Hematol 17:31–35

1065-6251

Introduction

Neutrophils play a critical role in the early stages of the inflammatory response to bacteria and fungi infection by ingesting and killing invading pathogens through the action of proteolytic enzymes, antimicrobial proteins, and reactive oxygen species [1]. This role is illustrated by the increased susceptibility to infection and sepsis of individuals with congenital or acquired neutropenia or defects of neutrophil functions [2]. Neutrophils are also involved in wound healing and in fine-tuning immune response by virtue of their ability to produce a number of cytokines and chemokines such as tumor necrosis factor α (TNFα), interleukin-1, interleukin-12, transforming growth factor β (TGF-β), interleukin-8, growth related oncogene α (GRO-α), macrophage inflammatory protein 1 α/β (MIP-1α/β), MIP-3α/β, and interferon-γ inducible protein 10 [3•]. Neutrophils are short-lived cells (12–72 h). As a first-line of defense against host insult, they are rapidly and massively recruited from the circulation into inflammatory sites, where their survival is regulated by a complex array of factors and mechanisms. The present review addresses recent finding related to the pathways responsible for the control of neutrophil survival.

Apoptosis

The present paradigm supports that apoptosis is the predominant cell death pathway in the neutrophil. The term apoptosis, proposed by Kerr in 1972, describe a morphological pattern of cell death characterized by cell shrinkage, nuclear chromatin condensation, DNA fragmentation into nucleosome-length fragments followed by chromatin condensation, and exposure of phosphatidylserine on the outer leaflet of the plasma membrane [4••]. All of these changes are related to the activation of caspases (cysteinyl aspartate proteases), an evolutionarily conserved family of proteins that irreversibly commit a cell to die [5•]. Thirteen caspases have been identified in the humans and seven of them are involved in apoptosis. According to their role in apoptosis, these caspases are classified as initiators (caspase-2, 8, 9, and 10) and effectors (caspase-3, 6, and 7), which kill cells by cleaving a number of cellular components. A phylogenetically distinct class of caspases (caspase-1, 4, 5, 11, and 12) mediate the proteolytic

processing of the precursors of the inflammatory cytokines interleukin-1 and interleukin-18 thus resulting in the induction of inflammatory responses [5•,6].

The activity of caspases is under the control of a family of proteins namely inhibitors of apoptosis proteins (IAPs), including cellular IAP 1 (c-IAP-1), c-IAP-2, X-linked IAP (XIAP), IAP-like protein 2 (ILP-2), neuronal apoptosis inhibitor protein (NAIP), melanoma inhibitor of apoptosis protein (ML-IAP), apollon and survivin, which bind and inhibit caspases 3, 9, and 7 [7]. Human neutrophils express c-IAP-1, c-IAP-2, and XIAP. The expression of cIAP-2 is low in aging neutrophils but increases by granulocyte colony-stimulating factor (G-CSF)-treatment, either *in vitro* or *in vivo* [8]. Neutrophils from septic patients express high levels of XIAP that may explain, at least partially, their delayed apoptotic rate [9]. Altznauer *et al.* [10] showed that only immature neutrophils express high levels of survivin. Interestingly, mature neutrophils re-express survivin after stimulation by G-CSF or granulocyte macrophage-colony stimulating factor (GM-CSF) and during the course of inflammatory diseases such as acute appendicitis, ulcerative colitis, and cystic fibrosis. Moreover, antisense specific inhibition of survivin expression in mature neutrophils impairs the antiapoptotic effects mediated by G-CSF and GM-CSF, supporting that the pro-survival action of both cytokines depends not only on their ability to increase the ratio between antiapoptotic and proapoptotic members of the B-cell lymphoma (Bcl-2) family of proteins.

There are two main pathways leading to caspase activation and induction of apoptosis: a death receptor or extrinsic pathway and a mitochondrial or intrinsic pathway. Both pathways involve the sequential activation of caspases in two distinct but converging pathways.

Regulation of neutrophil apoptosis via death receptors: the extrinsic apoptosis pathway

Death receptors include Fas, TNF receptor-1 (TNFR1), and TNF-related apoptosis-inducing ligand (TRAIL) receptors that transmit apoptotic signals after stimulation by specific ligands: Fas ligand (FasL), TNFα, and TRAIL, respectively. Stimulation of death receptors leads to the activation of the initiator caspase-8 that activates effector caspases leading to apoptotic cell death [11]. The role of death receptor in the control of neutrophil survival has been a matter of controversy. Because cross-linking of Fas receptors by agonistic antibodies directed to Fas results in a dramatic acceleration of apoptosis it has been suggested that the Fas/FasL system plays a major role in the regulation of spontaneous apoptosis of neutrophils [12]. However, blocking antibodies directed to Fas or agents able to block FasL are unable to increase neutrophil survival. Consistent with this observation, neutrophils from Fas (*lpr*) or FasL (*gld*) deficient mice display a normal rate of apoptosis supporting that apoptosis of resting neutrophils is not under the regulation of the Fas/FasL system [13]. Interestingly, Jonsson *et al.* [14] found that the ongoing inflammatory processes observed in immune complex-mediated inflammatory arthritis and thioglycollate-induced peritonitis require the forkhead transcription factor forkhead box O3 (Foxo3a), which acts by suppressing the expression of FasL thus promoting neutrophil viability at the inflammatory sites. This suggests that the Fas/FasL system controls survival of inflammatory neutrophils. Moreover, it has been recently reported that the Fas/FasL pathway is associated with the acceleration of neutrophil apoptosis in children with idiopathic neutropenia [15•].

The interaction of TNFα with TNFR1 may result in either the activation of nuclear factor (NF)-κB, promoting neutrophil survival, or the stimulation of apoptosis via recruitment of TNFR1-associated death domain protein (TRADD) and caspase-8. Shedding light on these contrasting effects, Micheau and Tschopp [16] have shown that activation of TNFR1 leads to the assembly of a membrane-bound complex, complex I, which involves TNFR1, TRADD, receptor interacting protein (RIP), and TNF receptor-associated factors (TRAFs). This complex is able to trigger NF-κB activation promoting neutrophil survival. In a second step, TRADD dissociates from TNFR1 and associates with Fas-associated protein with death domain (FADD) and caspase-8, leading to the assembly of a cytoplasmic complex, complex II, which induces the activation of caspases and cell death. Of note, the ability of TNFR1 to promote cell survival or cell death depending on the regulated assembly of complex I and complex II might explain contrasting results reported on the action of TNFα on neutrophil apoptosis. As expected, TNFα might also affect the fate of neutrophils at inflammatory sites by additional mechanisms. For example, TNFR1 has been shown to disrupt antiapoptosis pathways induced by survival factors in neutrophils [17], whereas it strongly impairs the depuration of apoptotic neutrophils by macrophages thus promoting inflammatory reactions [18•].

Unlike other cytokines of the TNF family, TRAIL is able to interact with a complex system of receptors that include two proapoptotic death receptors, TRAIL-R1 and TRAIL-R2, and three decoy receptors devoid of functional death domains [19•]. Under appropriate stimulatory conditions (treatment with interferon-α or interferon-γ) neutrophils produce high levels of TRAIL [20]. Interestingly, TRAIL does not appear to be involved in the control of apoptosis of freshly isolated neutrophils, but promotes the apoptosis and clearance of senescent neutrophils by a mechanism dependent on the interaction of stromal cell-derived factor 1 (SDF-1) with chemokine (C-X-C motif) receptor 4 (CXCR4), which induces both the production

of TRAIL and the expression of TRAIL death receptors [21].

Regulation of apoptosis via the mitochondria: the intrinsic apoptosis pathway

The mitochondrial pathway of apoptosis occurs via the activation of caspase-9 as an initiator caspase and involves the Bcl-2 family members. This pathway is triggered by a variety of stressors such as DNA damage, growth factor deprivation, cytoskeleton damage, endoplasmic reticulum stress, detachment from the cell matrix (anoikis), inhibition of macromolecular synthesis, chemotherapy drugs, and γ-irradiation. These stimuli lead to the permeabilization of the mitochondrial outer membrane and the release of cytochrome c and other proapoptotic proteins such as second mitochondria-derived activator of caspase/direct IAP-binding protein with low isoelectric point (pI) (Smac/DIABLO), apoptosis-inducing factor (AIF), and Omi/high temperature requirement protein A2 (Omi/HtrA2) to the cytosol. Cytochrome c then interacts with the tryptophan-aspartate (WD) repeat domain of apoptotic protease activating factor 1 (APAF-1), leading to the oligomerization of Apaf-1 and the formation of the apoptosome, which recruits caspase-9 and activates effector caspases 3, 6, and 7 [22]. The Bcl-2 family proteins act as prominent gatekeepers controlling the release of cytochrome c and other proapoptotic proteins from the mitochondria to the cytosol. Three subfamilies of Bcl-2 proteins have been characterized on the basis of function and sequence similarity. The antiapoptosis subfamily comprises Bcl-2 itself, Bcl-x_L, Bcl-w, myeloid cell leukemia sequence (Mcl-1), A1, and Bcl-B. The proapoptosis proteins include two subfamilies, the multidomain subfamily represented by Bax and Bak, and the 'BH3-only' subfamily represented by Bim, Bad, Bid, Bik, Bmf, Puma, Noxa, and Hrk [23].

Neutrophils contain relatively few mitochondria, however they form a highly developed network [24]. The role of mitochondria in neutrophil physiology has been clarified in the last years. Mitochondria hardly contribute to the energy status of neutrophils, which is mainly dependent on a high rate of glycolysis [25]. However, even though neutrophil mitochondria contain low levels of cytochrome c, they appear to play a critical role in the control of neutrophil survival. It has been proposed that the intrinsic pathway of neutrophil apoptosis has a lowered threshold requirement for cytochrome c which is compensated by an increased expression of Apaf-1 and other proapoptotic proteins such as Smac/DIABLO and HtrA2/Omi, that can be rapidly and massively released from the mitochondria into the cytosol after appropriate stimulation [26,27].

The balance between the expression of the proapoptotic and antiapoptotic members of the Bcl-2 family of proteins is thought to determine the lifespan of neutrophils. A prominent feature of neutrophils is their very short half-life and, consistent with this property, proapoptotic proteins of the Bcl-2 family such as Bax, Bak, Bad, Bid, and Bik are constitutively expressed in the neutrophil [27]. Neutrophils also express the antiapoptotic proteins Mcl-1 and A1/Bfl-1. Although there are some contradictory reports regarding the expression of Bcl-X_L at the protein level, Bcl-2 has not been detected in resting neutrophils [27,28•]. The evidence summarized in Table 1 [29–40] strongly suggests that the mitochondrial pathway of apoptosis plays a critical role in the spontaneous apoptosis of neutrophils and also modulate the survival of activated cells.

NETosis

NETosis is a novel neutrophil death pathway triggered upon neutrophil activation that leads to the release of neutrophil extracellular traps (NETs) that are able to kill

Table 1 Role of the intrinsic pathway in the control of neutrophil survival

Experimental model	Observation	Reference
$Bak^{-/-}$ $Bax^{-/-}$ DKO mice	Neutrophilia	[29]
Bax deficient neutrophils	Delayed spontaneous apoptosis	[30]
Neutrophils + GM-CSF	Low Bax levels and delayed apoptosis	[31]
Neutrophils + TNFα or *Mycobacterium tuberculosis*	Increased Bax/Bcl-XL ratio and accelerated apoptosis	[32]
Neutrophils + antiapoptotic stimuli (GM-CSF, IL-8, LPS)	Blockade of proapoptotic activity of Bad by Ser-phosphorylation	[33]
Neutrophils + pro-apoptotic stimuli (Nicotinic acid)	Dephosphorylation of Bad	[34]
Bim-deficient neutrophils	Delayed apoptosis in response to cytokine withdrawal and cytotoxic drugs	[35]
Neutrophils + calcium pyrophosphate crystals	Low Bim levels and delayed apoptosis	[36]
A1-deficient neutrophils	Accelerated spontaneous apoptosis	[37]
Neutrophils + LPS or GM-CSF	Increased levels of A1 and delayed apoptosis	[38]
Mcl-1-deficient neutrophils	Accelerated spontaneous apoptosis	[39]
$Mcl\text{-}1^{-/-}$ mice	Marked reduction in the number of neutrophils in the blood, spleen, and peritoneal exudates	[40]

Bcl-XL, B-cell lymphoma X_L; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; LPS, lipopolysaccharide; Mcl-1, myeloid cell leukemia sequence.

a variety of bacteria, fungi, and parasites [41,42]. NETs are webs formed by chromatin and granule proteins that provide a high local concentration of antimicrobial molecules. This pathway of neutrophil death depends on the activation of the respiratory burst and it is characterized by a number of specific morphological changes different from those described in apoptotic and necrotic neutrophils, such as disintegration of the nuclear envelope, mixing of cytoplasmic and nuclear materials, and loss of internal membranes and cytoplasmic organelles, thus defining a novel cell death pathway [41,42]. Interestingly, following priming with GM-CSF and subsequent stimulation by complement component C5a (C5a), viable neutrophils release NETs containing mitochondrial, but not nuclear DNA. Strikingly, release of NETs by neutrophils stimulated by GM-CSF and C5a was associated with an increased cell survival compared with resting neutrophils [43••]. This clearly indicates that the production of NETs does not always require neutrophil death. Production of NETs by neutrophils seems to play an important role not only in host defense against infection [41,42,44•] but also in autoimmunity. It has been recently reported that NETs are released by antineutrophil cytoplasm autoantibodies (ANCA)-stimulated neutrophils and triggers both vasculitis and the autoimmune response against neutrophil components observed in patients with small-vessel vasculitis [45••].

Autophagic cell death

Autophagy represents an evolutionarily conserved catabolic pathway that enables eukaryotic cells to degrade and recycle cellular components. Autophagic cell death is a programmed cell death different from apoptosis, characterized by the formation of multilamellar autophagosomes under the control of GTPases and phosphatidylinositol kinases that engulf intracellular components [46]. Interestingly, the pathways of autophagic cell death and apoptosis are interconnected. Both pathways share several genes and a variety of stimuli are able to activate either pathway [47••]. Recent studies support that autophagic cell death is operative in the neutrophil. In this regard, it has been shown that sialic acid-binding immunoglobulin-like lectin 9 (Siglec-9) ligation on the neutrophil surface induces cell death. Of note, death induction by Siglec-9 is enhanced when neutrophils were exposed to proinflammatory antiapoptotic cytokines, such as GM-CSF, interferon-α, or interferon-γ and also in neutrophils obtained from patients with acute septic shock or rheumatoid arthritis [48,49]. Cell death was largely caspase-independent, required the activation of the respiratory burst, and it was characterized by cytoplasmic vacuolization and other nonapoptotic morphologic features closely resembling autophagic cell death [48,49]. A similar mechanism of cell death was described for neutrophils stimulated by TNFα in the presence of the caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (z-VAD-fmk) [50]. Interestingly, it has been recently reported that human intravenous immunoglobulin (IVIg) preparations contain natural anti-Siglec-9 autoantibodies, which are able to ligate Siglec-9 on neutrophils and induce autophagic-like cell death in the presence of GM-CSF and some other survival cytokines [51].

Conclusion

As the Nomenclature Committee on Cell Death 2009 stated [4••], different types of cell death are often defined by morphological criteria, without a clear reference to precise biochemical mechanisms. Moreover, there are numerous examples in which cells show mixed features, with signs of both apoptosis and necrosis. This made it difficult to define how the survival of neutrophil is regulated under physiological and pathological conditions. However, it is clear that the half-life of neutrophils is heavily affected in the course of inflammatory and autoimmune conditions. In fact, neutrophils have been shown to display a prolonged half lifespan in patients with endotoxemia, traumatic injury, pneumonia, systemic inflammatory response syndrome (SIRS), inflammatory bowel disease, and rheumatoid arthritis. By contrast, a decreased neutrophil survival was reported for patients with glycogen storage disease type 1b, ANCA-associated vasculitis, and systemic lupus erythematosus [52•]. Understanding the molecular pathways that control neutrophil survival holds therapeutic promise in a variety of infectious, inflammatory, and autoimmune diseases.

Acknowledgement

The present study was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, the Buenos Aires University School of Medicine, and the Agencia Nacional de Promoción Científica y Tecnológica (Argentina).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 72).

1 Segal AW. How neutrophils kill microbes. Annu Rev Immunol 2005; 23:197–223.

2 Skokowa J, Germeshausen M, Zeidler C, *et al.* Severe congenital neutropenia: inheritance and pathophysiology. Curr Opin Hematol 2007; 14:22–28.

3 • Cowburn AS, Condliffe AM, Farahi N, *et al.* Advances in neutrophil biology: clinical implications. Chest 2008; 134:606–612.
This review provides an excellent summary of recent advances in the field of neutrophil physiology.

4 •• Kroemer G, Galluzzi L, Vandenabeele P, *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009; 16:3–11.
In this article, the Nomenclature Committee on Cell Death (NCCD) proposes unified criteria for the definition of cell death and of its different morphologies, although formulating several caveats against the misuse of words and concepts that slow down progress in the area of cell death research.

5 • Pop C, Salvesen GS. Human caspases: activation, specificity, and regulation. J Biol Chem 2009; 284:21777–21781.
This recent review provides a very good summary on the function of caspases.

6 Scott AM, Saleh M. The inflammatory caspases: guardians against infections and sepsis. Cell Death Differ 2007; 14:23–31.

7 Vaux DL, Slike J. IAPs, RINGs and ubiquitylation. Nat Rev Mol Cell Biol 2005; 6:287–297.

8 Hasegawa T, Suzuki C, Sakamoto K, *et al.* Expression of the inhibitor of apoptosis (IAP) family members in human neutrophils: up-regulation of cIAP2 by granulocyte colony-stimulating factor and overexpression of cIAP2 in chronic neutrophilic leukemia. Blood 2003; 101:1164–1171.

9 O'Neill AJ, Doyle BT, Molloy E, *et al.* Gene expression profile of inflammatory neutrophils: alterations in the inhibitors of apoptosis proteins during spontaneous and delayed apoptosis. Shock 2004; 21:512–518.

10 Altznauer F, Martinelli S, Yousefi S, *et al.* Inflammation-associated cell cycle: independent block of apoptosis by survivin in terminally differentiated neutrophils. J Exp Med 2004; 199:1343–1354.

11 Guicciardi ME, Gores GJ. Life and death by death receptors. FASEB J 2009; 23:1625–1637.

12 Liles WC, Kiener PA, Ledbetter JA, *et al.* Differential expression of Fas (CD95) and FasL on normal human phagocytes: implications for the regulation of apoptosis in neutrophils. J Exp Med 1996; 184:429–440.

13 Fecho KL, Cohen PL. Fas ligand (gld) and Fas (lpr) deficient mice do not show alterations in the extravasation or apoptosis of inflammatory neutrophils. J Leuk Biol 1998; 64:373–383.

14 Jonsson H, Allen P, Peng SL. Inflammatory arthritis requires Foxo3a to prevent Fas ligand-induced neutrophil apoptosis. Nat Med 2005; 11:666–671.

15 • Nadeau KC, Callejas A, Wong WB, *et al.* Idiopathic neutropenia of childhood is associated with Fas/FasL expression. Clin Immunol 2008; 129:438–447.
This article supports that an enhanced expression of the Fas/FasL system might be involved in the etiopathogenesis of idiopathic neutropenia.

16 Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003; 114:181–190.

17 Daigle I, Yousefi S, Colonna M, *et al.* Death receptors bind SHP-1 and block cytokine-induced antiapoptotic signaling in neutrophils. Nat Med 2002; 8:61–67.

18 • Michlewska S, Dransfield I, Megson IL, *et al.* Macrophage phagocytosis of apoptotic neutrophils is critically regulated by the opposing actions of pro-inflammatory and anti-inflammatory agents: key role for TNF-alpha. FASEB J 2009; 23:844–854.
This article reveals a new mechanism through which TNFα might contribute to the perpetuation of inflammatory responses.

19 • Mahalingam D, Szegezdi E, Keane M, *et al.* TRAIL receptor signaling and modulation: are we on the right TRAIL? Cancer Treat Rev 2009; 35:280–288.
This recent review provides a good overview on the expression and function of TRAIL receptors.

20 Cassatella MA. On the production of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) by human neutrophils. J Leuk Biol 2006; 79:1140–1149.

21 Lum JJ, Bren G, McClure R, *et al.* Elimination of senescent neutrophils by TNF-related apoptosis-inducing ligand. J Immunol 2005; 175:1232–1238.

22 Hinds MG, Day CL. Regulation of apoptosis: uncovering the binding determinants. Curr Opin Struct Biol 2005; 15:690–699.

23 Adams JM, Cory S. Bcl-2-regulated apoptosis: mechanisms and therapeutic potential. Curr Opin Immunol 2007; 19:1–9.

24 Fossati G, Moulding DA, Spiller DG, *et al.* The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. J Immunol 2003; 170:1964–1972.

25 Van Raam BJ, Sluiter W, de Wit E, *et al.* Mitochondrial membrane potential in human neutrophils is maintained by complex III activity in the absence of supercomplex organisation. PLoS One 2008; 3:e2013.

26 Maianski NA, Geissler J, Srinivasula SM, *et al.* Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. Cell Death Differ 2004; 11:143–153.

27 Van Raam BJ, Verhoeven AJ, Kuijpers TW. Mitochondria in neutrophil apoptosis. Int J Hematol 2006; 84:199–204.

28 • Kennedy AD, DeLeo FR. Neutrophil apoptosis and the resolution of infection. Immunol Res 2009; 43:25–61.
This study provides an excellent overview of the multiple mechanisms involved in the control of neutrophil survival.

29 Lindsten T, Ross AJ, King A, *et al.* The combined functions of proaptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol Cell 2000; 6:1389–1399.

30 Gardai SJ, Hildeman DA, Frankel SK, *et al.* Phosphorylation of Bax Ser^{184} by Akt regulates its activity and apoptosis in neutrophils. J Biol Chem 2004; 279:21085–21095.

31 Dibbert B, Weber M, Nikolaizik WH, *et al.* Cytokine-mediated Bax deficiency and consequent delayed neutrophil apoptosis: a general mechanism to accumulate effector cells in inflammation. Proc Natl Acad Sci USA 1999; 96:13330–13335.

32 Perskvist N, Long M, Stendahl O, *et al.* Mycobacterium tuberculosis promotes apoptosis in human neutrophils by activating caspase-3 and altering expression of Bax/Bcl-xL via an oxygen-dependent pathway. J Immunol 2002; 168:6358–6365.

33 Francois S, El BJ, Dang PM, *et al.* Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF-κB signaling pathways leading to increased levels of Mcl-1, A1, and phosphorylated Bad. J Immunol 2005; 174:3633–3642.

34 Kostylina G, Simon D, Fey MF, *et al.* Neutrophil apoptosis mediated by nicotinic acid receptors (GPR109A). Cell Death Differ 2008; 15:134–142.

35 Villunger A, Scott P, Bouillet P, *et al.* Essential role for the BH3-only protein Bim but redundant roles for Bax, Bcl-2, and Bcl-w in the control of granulocyte survival. Blood 2003; 101:2393–2400.

36 Higo T, Duronio V, Tudan C, *et al.* Calcium pyrophosphate dihydrate crystal-induced inhibition of neutrophil apoptosis: involvement of Bcl-2 family members. Inflamm Res 2009 [Epub ahead of print].

37 Hamasaki A, Sendo K, Nakayama N, *et al.* Accelerated neutrophil apoptosis in mice lacking A1-a, a subtype of the bcl-2 related A1 gene. J Exp Med 1998; 188:1985–1992.

38 Kotani J, Avallone NJ, Lin E, *et al.* Fas-mediated neutrophil apoptosis and associated A1 protein expression during systemic inflammation are regulated independently of both tumor necrosis factor receptors. Shock 2003; 19:201–207.

39 Moulding DA, Quayle JA, Hart CA, *et al.* Mcl-1 expression in human neutrophils: regulation by cytokines and correlation with cell survival. Blood 1998; 92:2495–2502.

40 Dzhagalov I, St John A, He YW. The antiapoptotic protein Mcl-1 is essential for the survival of neutrophils but not macrophages. Blood 2007; 109:1620–1626.

41 Fuchs TA, Abed U, Goosmann R, *et al.* Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 2007; 176:231–241.

42 Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. Nat Rev Microbiol 2007; 5:577–582.

43 •• Yousefi S, Mihalache C, Kozlowski E, *et al.* Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. Cell Death Differ 2009; 16:1438–1444.
This article reveals the ability of human neutrophils to release mitochondrial DNA to form NETs without affecting cell viability.

44 • Gulmaraes-Costa AB, Nascimento MTC, Froment GS, *et al.* Leishmania amazonensis promastigotes induce and are killed by neutrophil extracellular traps. Proc Natl Acad Sci USA 2009; 106:6748–6753.
This article supports the notion that NETs play an important role, not only in immunity against bacteria and fungi infections, but also in the immune response against parasites.

45 •• Kessenbrock K, Krumbholz M, Schonermarck U, *et al.* Netting neutrophils in autoimmune small-vessel vasculitis. Nature Med 2009; 15:623–625.
This study shows that NETs are involved not only in antimicrobial immunity, but also in the development of autoimmune processes such as small-vessel vasculitis.

46 Galluzi L, Morselli E, Vicencio JM, *et al.* Life, death and burial: multifaceted impact of autophagy. Biochem Soc Trans 2008; 36:786–790.

47 •• Eisenberg-Lerner A, Bialik S, Simon HU, *et al.* Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ 2009; 16:966–975.
This review provides an excellent summary on the evidences supporting the crosstalk between two different cell death pathways; apoptosis and autophagy.

48 Von Gunten S, Yousefi S, Seitz M, *et al.* Siglec-9 transduces apoptotic and nonapoptotic death signals into neutrophils depending on the proinflammatory cytokine environment. Blood 2005; 106:1423–1431.

49 Von Gunten S, Simon HU. Sialic acid binding immunoglobulin-like lectins may regulate innate immune response by modulating the life span of granulocytes. FASEB J 2006; 20:601–605.

50 Maianski NA, Roos D, Kuijpers TW. Tumor necrosis factor induces a caspase-independent death pathway in human neutrophils. Blood 2003; 101:1987–1995.

51 von Gunten S, Simon HU. Natural anti-Siglec autoantibodies mediate potential immunoregulatory mechanisms: implications for the clinical use of intravenous immunoglobulins (IVIg). Autoimmun Rev 2008; 7:453–456.

52 • Luo HR, Loison F. Constitutive neutrophil apoptosis: mechanisms and regulation. Am J Hematol 2008; 83:288–295.
This review provides an excellent summary of recent advances directed to define how the survival of neutrophils is regulated in health and disease.