Introduction

I am pleased to present this Special Issue of *Acta Haematologica* on the topic of Apoptosis. Since the description of apoptosis by Wyllie, Kerr, and Currie three decades ago [1], the field has expanded both in breadth (description of the phenomenon in different systems), depth (detailed analysis of the molecular events), and significance (recognition of its role in many of the fundamental biological processes of multicellular organisms, including development, differentiation, immune response, and tissue homeostasis). While some of the earliest key findings derived from hematology, such as the discovery of Bcl-2 [2], more recent advances have revealed a role for dysregulated apoptosis in many hematological disorders. It is our hope that this series of review articles will serve to apprise the hematologist of the basic mechanisms of apoptosis as well as to point out its relevance to clinical conditions.

The first article by Festjens et al. provides a detailed discussion of the mitochondria and their central role in apoptosis. This will provide a basis for understanding many of the observations described in subsequent papers. Signals for apoptosis often come from the outside, and the second paper, by Andrade et al. describes the role of Granzyme B in triggering apoptosis. Signaling through Fas and the tumor necrosis factor- α receptor (TNFR) are also briefly discussed but have been reviewed elsewhere [3]. Bcl-2 and related family members play a central role in apoptosis, and their role in the lymphoid system is described by Ivanovska et al. We then turn our attention to the unique features of apoptosis in PMN described by Maianski et al. and to the role of apoptosis in platelet biogenesis as explained by Kaluzhny and Ravid. Finally, we shift our attention to the role of apoptosis in pathologic processes, including the myelodysplastic syndrome, dis-

cussed by Parker and Mufti, and myeloid leukemia, reviewed by Boyapati et al. In the last article, the knowledge of apoptotic mechanisms guides new therapeutic approaches in leukemia, as outlined by Adachi et al.

Before leaping into the myriad of details, a brief overview of apoptosis is warranted. All multicellular organisms, including plants, possess machinery to allow individual cells to self-destruct in a way that minimizes inflammation or damage to bystander cells. This cell-autonomous system is known as programmed cell death, or apoptosis, and key features are conserved from *Caenorhabditis elegans* to man. These central features include caspases, which are intracellular proteases that have a cysteine in the active site and cleave proteins after an aspartic acid residue within a consensus sequence. They exist as proenzymes that are proteolytically processed to yield a p20 and p10 heterodimer, and the active caspase consists of two heterodimers. Mammals possess a number of caspases that function either as upstream triggers or as downstream effectors. The upstream caspases, such as caspase 8, contain a large prodomain that associates with an adaptor molecule that serves to bring two caspase molecules together to allow proximity-induced processing. Receptor occupancy, as in the case of Fas or TNFR, causes receptor aggregation, recruitment of adaptor proteins such as FADD (Fas-associated death domain) or TRADD (TNF receptor-associated death domain), and subsequent activation of caspase 8 or 10. The activated signaling caspase can then amplify the apoptotic signal through cleavage of a downstream caspase such as caspase 3, or can activate the mitochondrial pathway (see below). Caspase 3 is readily activated by proteolysis and once activated has a high turnover number thereby earning its designation as an effector caspase.

A second key element in apoptosis is the participation of a Bcl-2 family member. The Bcl-2 family consists of three subfamilies: the *Bcl-2 subfamily*, which is anti-apoptotic; the *Bax subfamily*, which is pro-apoptotic and includes Bax and Bak, and the *BH3 subfamily*, which collaborates with a Bax family member to trigger apoptosis. The Bax family shares homology with the Bcl-2 family in three domains, but lacks the fourth – anti-apoptotic – domain. The BH3 proteins share homology only in domain 3 (hence the name, Bcl-2 Homology domain 3). These proteins serve as sensors of intracellular discontent. Dysregulated proteolysis, imbalance of kinases and phosphatases, and disruption of the cytoskeleton can all trigger activation of a BH3 family member to translocate to the mitochondria and interact with Bax or Bak. The effect of BH3 and Bax family interaction is to trigger mitochondrial alterations that culminate in the release of mitochondrial intermembrane space proteins including cytochrome *c*. Cytochrome *c*, once in the cytosol, interacts with another conserved element of apoptosis, Apaf-1. Apaf-1 binds cytochrome *c*, ATP or dATP, and recruits procaspase 9 and effector caspases such as caspase 3. This large complex, termed the apoptosome, results in activation of caspase 9, which in turn cleaves and activates the effector caspases including caspase 3. Caspase 3 cleaves a variety of targets including proteins that would otherwise signal cell survival, and also cleaves DNA fragmentation factor to release an endonuclease (Caspase-activated DNase), thus ensuring destruction of the genome and loss of replicative potential of the cell. In the case of viral infection, this process ensures destruction of the viral genome as well.

Apoptosis is characterized by a number of additional morphologic and biochemical features, including externalization of phosphatidylserine, which invites phagocytes to ingest the dying cell, as well as loss of cellular ions and water, resulting in dramatic acidification and shrinkage. Nuclear condensation and fragmentation are due to chromatin cleavage as well as proteolysis of nuclear lamins. The membrane skeleton is made rigid by the activation of intracellular tissue transglutaminases which cross-link proteins to form a stable meshwork that limits leak-

age of cell contents and perhaps facilitates ingestion by phagocytes. The end result is the efficient dismantling and removal of unwanted cells.

Failure in any of these components can lead to the accumulation of unwanted cells, including those that have acquired mutations in their DNA and have begun the process of malignant transformation. In many cases, it is the loss of a pro-apoptotic regulator that is an essential first step, of which p53 is exemplary. Such an elegant system of cell suicide is not without checks and balances, and for every factor named thus far, there are an equal or larger number of proteins that oppose the process in order to maintain cell survival. For example, Fas signaling can be attenuated by the expression of FLIP, which displaces caspase 8 from the Fas/FADD complex. Release of mitochondrial factors is held in check by Bcl-2 or Bcl-xL. Caspase activation is suppressed by Inhibitor of Apoptosis Proteins (IAPs). Failure to suppress apoptosis can also lead to problems, as discussed in the context of myelodysplasia.

As our understanding of apoptosis in physiology and pathophysiology evolves, and as new therapies are introduced that target specific aspects of apoptosis, it will become increasingly necessary to understand this process in molecular detail. It is hoped that this series of reviews will smooth the progress of hematologists and oncologists towards that objective.

Roberta A. Gottlieb, La Jolla, Calif.

References

- 1 Wyllie AH, Kerr JF, Currie AR: Cell death in the normal neonatal rat adrenal cortex. *J Pathol* 1973;111:255–261.
- 2 Hockenbery D, Nunez G, Millman C, Schreiber RB, Korsmeyer SJ: Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 1990;348:334–336.
- 3 Greil R, Anether G, Johrer K, Tinhofer I: Tracking death dealing by Fas and TRAIL in lymphatic neoplastic disorders: Pathways, targets and therapeutic tools. *J Leukoc Biol* 2003; 74:311–330.