Programmed cell death (apoptosis) and the resolution of systemic inflammation

John C. Marshall, MD; R. William G. Watson, PhD

. . . To die to sleep;
No more; and, by a sleep to say we end
The heartache and the thousand natural shocks
That flesh is heir to, 'tis a consummation
Devoutly to be wish'd....

Hamlet Act II, scene 3

The prospect of death held considerable attraction to Shakespeare's melancholy Dane; however, for the clinician or scientist, death has traditionally been regarded as the ultimate expression of biologic failure. The significance of the ultimate demise of the entire organism is more appropriate fodder for philosophers and theologians. But the controlled death of cells has recently become a subject of intense interest to investigators in a variety of disciplines, with the recognition that in the living host death is a continuous process of fundamental importance to normal growth and development.[1]

In the embryo, for example, discrete anatomic structures, such as the interdigital web spaces or pharyngeal clefts, develop as a consequence of the programmed death of intervening tissues; failure of this process results in congenital abnormalities such as webbing of the fingers or craniofacial abnormalities. During immunologic maturation, lymphocytes that react to host antigens are selectively eliminated through a process of programmed cell death; failure of this process leads to autoimmune disease. Transformed cells are recognized by immune cells and induced to die through a similar process, thus preventing the development of cancer. Epithelial cells of the skin or gastrointestinal tract that normally undergo rapid turnover, are shed after they have first been induced to die. And inflammatory cells such as neutrophils, whose numbers increase dramatically in response to an infectious challenge, disappear from the circulation because of the activation of a cell death program that leads to their rapid removal by phagocytic cells of the liver and spleen. The biologic process responsible for each of these cellular deaths is known as *apoptosis* or programmed cell death.

Apoptosis: an overview

Morphologic features

Apoptosis (pronounced a puh toe sis) is a tightly controlled process that proceeds so rapidly in vivo that it is not readily apparent on histologic examination. The cell undergoing apoptosis shows a characteristic pattern of changes (Fig. 1). The cytoplasm develops blebs, and specialized structures such as microvilli are lost. The cell ceases to adhere to surrounding cells of the extracellular matrix. Chromatin in the nucleus condenses and clumps. Fragmentation of strands of DNA can be seen on DNA gel electrophoresis: DNA is broken down into smaller strands, producing a characteristic ladder pattern on the gel (Fig. 1). The cell itself shrinks, and breaks down into membrane-bound fragments called apoptotic bodies that are recognized and phagocytosed by surrounding macrophages. The process takes less than an hour and, because the apoptotic cellular fragments do not evoke a host response, proceeds without any local inflammatory disruption. Apoptosis is strikingly different from necrosis, the more familiar form of cell death (Fig. 2) in which disruption of the cell membrane is an early feature. In necrosis, membrane damage results in spillage of intracellular contents into the surrounding cellular milieu, evoking an inflammatory response and resulting in injury to adjacent normal cells.[2]

Biochemical mechanisms and genetic regulation

Apoptosis is both highly controlled and highly conserved in evolutionary

From the Department of Surgery, Surgical Sepsis Laboratories, and the ICU Research Group, The Toronto Hospital, University of Toronto, Toronto, Ont.

Correspondence and reprint requests to: *Dr. John C. Marshall, Eaton North 9-234, The Toronto Hospital, General Division, 200 Elizabeth St., Toronto ON M5G 2C4; tel: 416 340-5204; fax: 416 595-9486; e-mail: jmarshall@torhosp.toronto.on.ca*

development, occurring not only in vertebrates and invertebrates but also in plants. The development of the phloem, the ductal system that transports water from the roots to the leaves, occurs through the apoptotic breakdown of plant cells. The fact that apoptosis is conserved across a wide variety of species of living organisms has been a boon to investigators trying to dissect its genetic and biochemical regulation.

Caenorhabditis elegans, despite its grandiose name, is an unprepossessing little nematode worm. The mature *C. elegans* grows from a single cell to become a creature comprising 1090 cells and during this maturation loses precisely 131 cells through apoptosis. The simplicity and reproducibility of this process has permitted the identification of *C. elegans* genes that regulate apoptosis.[3] Two genes — termed *ced-3* and *ced-4* — induce apoptosis, whereas *ced-9* inhibits it.

Identification of these genes triggered a search for similar or homologous genes in human cells. A homologue of the *ced-9* gene was identified in a B-cell lymphoma and termed *bcl-2* (from *B-c*ell *l*ymphoma).[4] As did its counterpart in *C. elegans*, *bcl-2* inhibited the normal expression of apoptosis, resulting in dysregulated cellular proliferation and lymphoma. Other *ced-9* structural homologues have been subsequently identified; some, like *bcl-2*, inhibit apoptosis, whereas others accelerate the process.[5] Bcl-2 protein appears to be found primarily in mitochondria, where it is involved in the regulation of cytochrome C and calcium transport.[6]

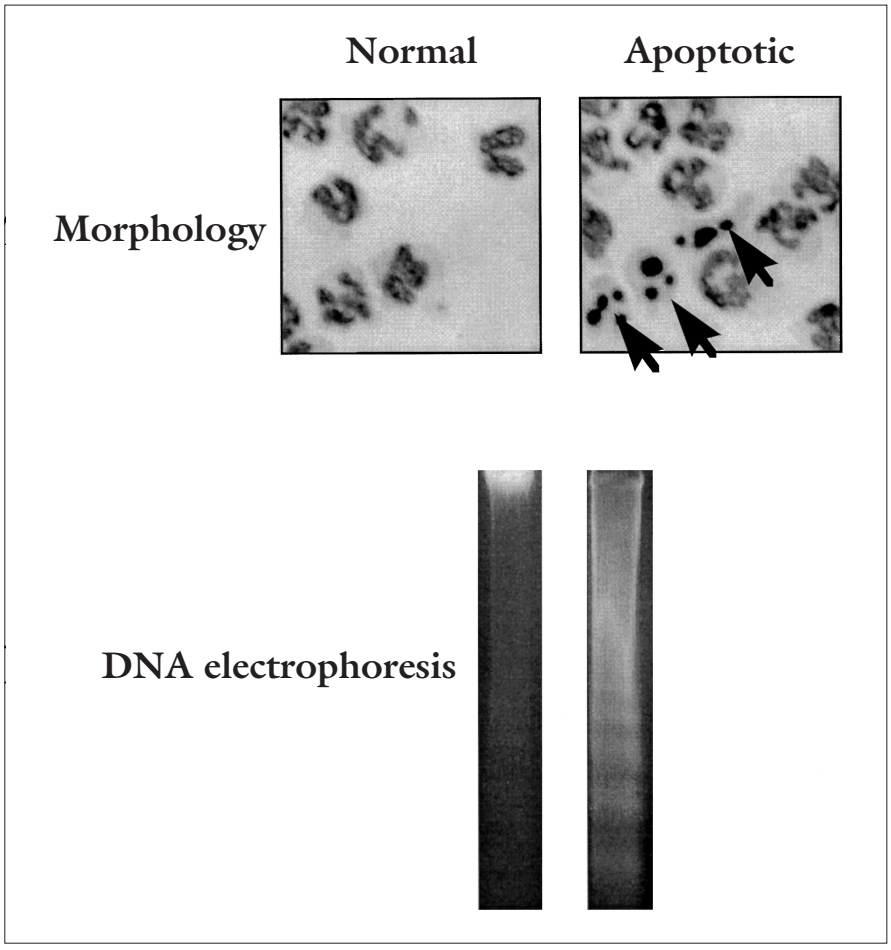

FIG. 1. The upper panel shows normal and apoptotic neutrophils as seen under the light microscope. Apoptotic cells (arrows) show nuclear condensation and cell shrinkage and are readily differentiated from viable cells. On DNA gel electrophoresis (bottom panel) apoptotic cells show a characteristic ladder pattern (right), the consequence of the fragmentation of DNA into fragments that are multiples of 180 base pairs.

The *C. elegans ced-3* gene demonstrated structural homology to an unexpected human gene: that for the interleukin-1β converting enzyme or ICE.[7] ICE is the enzyme responsible for cleaving pro-interleukin-1β (pro-IL-1β) into its active form, thus activating one of the principal mediators of systemic inflammation. Paradoxically, it is also the principal mediator of programmed cell death. ICE is one member of what is now recognized to be a family of death genes, collectively termed **caspases**. At least 10 members of this gene family have been identified.[8] They share a common enzymatic activity, namely the ability to cleave their target proteins at sites adjacent to the amino acid, aspartic acid. Their targets are many, including not only pro-IL-1β but also enzymes involved in DNA repair such as poly ADP ribose-polymerase, proteins such as actin that mediate cell structure and locomotion, and other enzymes of the caspase family. Human genes corresponding to *ced-4* have yet to be identified.

The expression of apoptosis in a given cell is thus a reflection of the dynamic interaction of proteins that trigger cell death and those that inhibit it. Numerous other proteins beyond those of the Bcl-2 and caspase families participate in apoptosis. The tumour suppressor gene, *p53*, for example, exerts its effects through its role in inducing apoptosis.[9]

Initiation and regulation

Cells can be induced to die an apoptotic death as a result of signals received from the environment. In fact, all cells have surface receptors whose engagement by the appropriate ligand will activate the cell death program. The first of these death receptors to be recognized is a protein known as Fas.[10] Fas can be activated through its interaction with a protein known as Fas ligand; the result of the binding of Fas ligand to cell-surface Fas is the induction of apoptosis. Fas, too, is but one of a family of cell-surface death receptors. One of the better known of this family is the smaller type 1 receptor for the cytokine tumour necrosis factor (TNF). Indeed TNF–TNF receptor interactions are responsible for the tumour killing effects of TNF; thus, TNF is probably more appropriately termed tumour apoptosis factor.

Surface receptors of the Fas and TNF receptor family form a complex with other cell membrane proteins involved in the activation of apoptosis (Fig. 3). Their engagement can lead to both cellular activation and cellular death by apoptosis. How the cell determines which of these contrasting effects will predominate is unknown but is an active area of ongoing study. The decision appears to hinge, at least in part, on whether or not a cytoplasmic protein involved in cellular activation, NF-κB, is activated.[11]

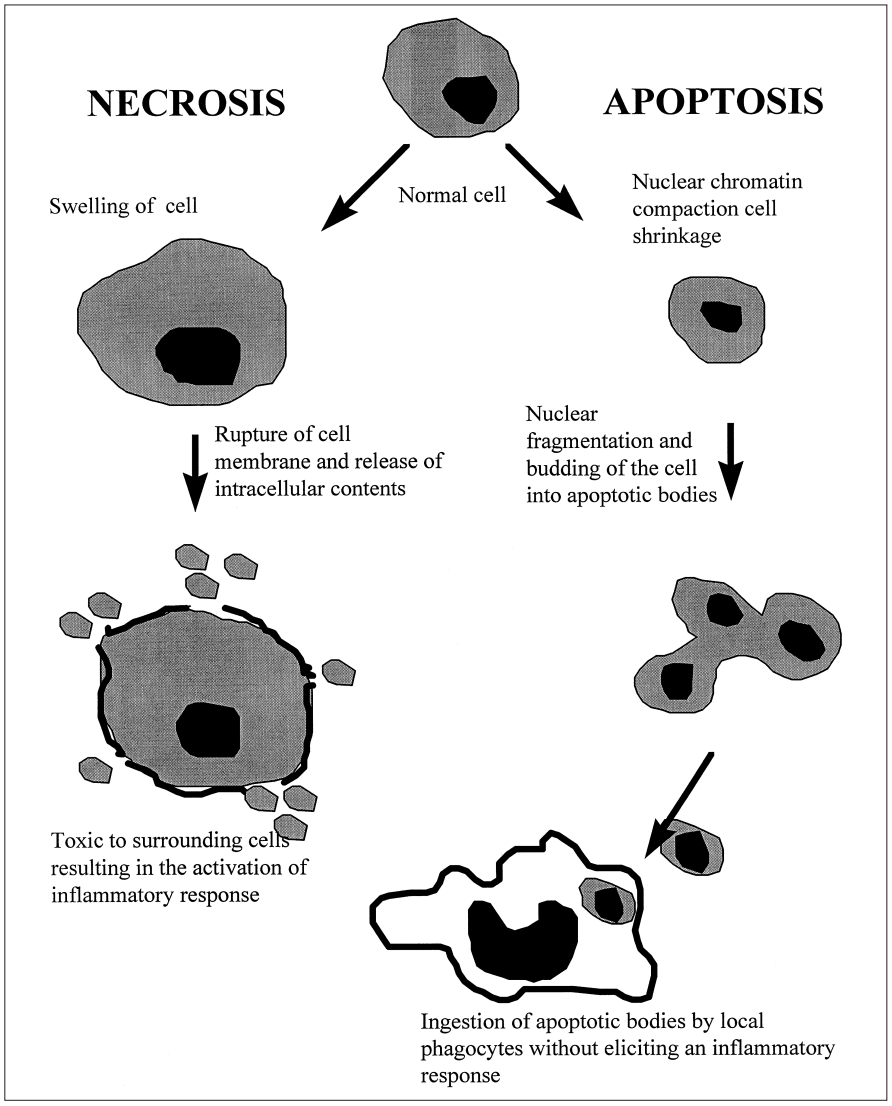

FIG. 2. Schematic diagram illustrating the basic differences between necrotic (left) and apoptotic (right) cell death.

Apoptosis and inflammatory disease

The ability to mount a rapid and effective response to an acute threat such as invasive infection or tissue injury is essential to survival. Yet the inflammatory response is nonselective in

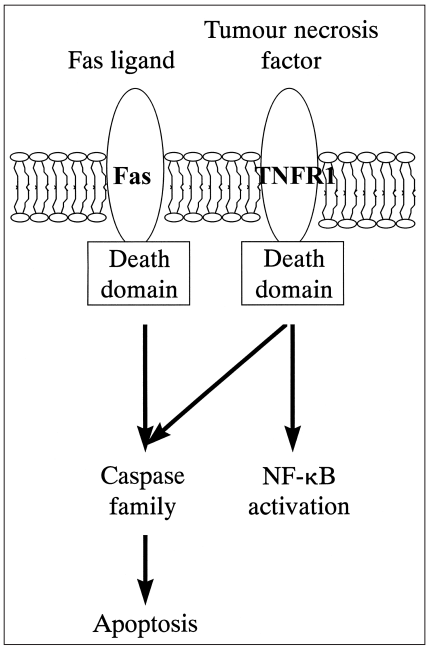

FIG. 3. Membrane receptors of the Fas family — particularly Fas and the receptor for tumour necrosis factor (TNF) — transmit signals from the environment to the interior of the cell that induce the cell to die an apoptotic death. Through mechanisms that are incompletely understood but of critical importance to the understanding of the regulation of inflammation, engagement of the TNF receptor (TNFR1) can both activate the cell and induce it to die an apoptotic death.

its targets, causing injury to invading organisms and host tissues alike.[12] It is therefore equally important that the host should possess efficient mechanisms to terminate an inflammatory response.[13] Inflammatory cell apoptosis is an important mechanism leading to the termination of inflammation; conversely, disorders of apoptosis result in diseases characterized by inappropriately expressed inflammation.

A number of inflammatory diseases arise as a consequence of accelerated apoptosis. Infusion of a soluble form of Fas into mice causes lethal hepatitis,[14] suggesting that certain cases of fulminant hepatic failure may result from inappropriate activation of hepatocyte apoptosis. Accelerated apoptosis is responsible for neuronal death in such diseases as Alzheimer disease and Huntington chorea, and for the elimination of CD4 cells following HIV infection. And a toxin produced by *Clostridium difficile* has been shown to induce apoptosis of colonic epithelial cells, providing an attractive explanation for the development of this fulminant colitis.[15]

Failure of apoptosis can also lead to abnormalities in the regulation of inflammation. A mouse strain lacking functional Fas death receptor develops an autoimmune disorder, resulting in arthritis and nephritis and reminiscent of systemic lupus erythematosus.[10] In humans, alterations in Fas lead to a systemic disorder characterized by lymphoproliferative disease and autoimmunity.[16] There is mounting evidence that failure of normal mechanisms regulating apoptosis of neutrophils contributes to the pathogenesis of the systemic abnormalities of trauma and sepsis.

Apoptosis and the termination of inflammation

Apoptosis is the predominant mechanism responsible for ending a neutrophil-mediated inflammatory process. The life span of the neutrophil is among the shortest of all cells. After their release from the bone marrow, neutrophils circulate for less than a day before they undergo a spontaneous apoptotic death and are phagocytosed by macrophages of the liver and spleen.[17] However, the expression of apoptosis in the neutrophil is exquisitely sensitive to signals received from the environment (Table I). The net effect of these influences in the setting of acute inflammation would appear to be prolongation of neutrophil survival as a result of the inhibition of apoptosis. For example, the β2 integrins, adhesion molecules on the neutrophil surface that play a key role in the adherence of the neutrophil to the vascular endothelium and its passage out of the circulation into a focus of inflammation, also transmit a signal to the nucleus that results in the inhibition of apoptosis.[18] Similarly, bacterial products such as endotoxin impede apoptosis as do host-derived proinflammatory mediators such as IL-1β or granulocyte colony stimulating factor.[19] Moreover, there is evidence that not only is the process of spontaneous neutrophil apoptosis delayed in inflammation, but neutrophils are also rendered refractory to death signals delivered through Fas or the TNF receptor.[20] Changes in the levels of intracellular antioxidant molecules may be responsible for these acute changes, since apoptosis can be induced by depletion of intracellular thiols.[21]

Inflammatory neutrophils, similar to transformed cells, survive for longer periods and lose their susceptibility to the mechanisms that normally induce cell death. Thus, the identification of signals that can cause the activated neutrophil to die an apoptotic death is important in understanding the mechanisms responsible for terminating an inflammatory response. To date, only one host cytokine,

Table I

Factors Modulating the Expression of Neutrophil Apoptosis

Mediators/processes	Retard apoptosis	Accelerate apoptosis
Soluble mediators	Interleukin-1β	Interleukin-6*
	Interleukin-2	Interleukin-10
	Interleukin-6*	Tumour necrosis factor*
	Granulocyte colony stimulating factor	Fas ligand
	Granulocyte macrophage colony stimulating factor	
	Interferon gamma	
	Tumour necrosis factor*	
	C5a	
	Endotoxin (lipopolysaccharide)	
	Glucocorticoids	
Cellular processes	β2 integrin adhesion	Phagocytosis of *Escherichia coli*
	Elevated intracellular calcium	L-selectin adhesion
		Reduced intracellular glutathione

*Contradictory data exist for these mediators.

interleukin-10, has been shown to reverse the block to programmed cell death that occurs in inflammation.[22]

Apoptosis in trauma and critical illness

Altered apoptosis appears to be responsible for some of the alterations in inflammatory and immune cell function seen after trauma and critical illness. Lymphopenia is a common finding in the victim of trauma or critical surgical illness. Studies in patients with major burn injury have shown increased rates of apoptosis in peripheral blood lymphocytes.[23] In contrast, however, neutrophils harvested from burn patients survive for prolonged periods in vitro, as a result of a delay in the expression of spontaneous apoptosis.[24]

We have studied the expression of neutrophil apoptosis in a cohort of patients who underwent major elective surgery (repair of an abdominal aortic aneurysm) or patients admitted to an intensive care unit who met clinical criteria for the systemic inflammatory response syndrome (SIRS).[25] Neutrophils harvested from both patient groups showed delays in apoptosis, and plasma from patients with SIRS could inhibit the expression of apoptosis in neutrophils from healthy volunteers (Dr. M.F. Jimenez, University of Toronto: unpublished data, 1997). Thus, a functional correlate of systemic inflammation is a state of neutrophil refractoriness to the normal processes of cellular suicide, and the process of cell death is emerging as a new target for therapeutic intervention in an attempt to limit the adverse sequelae of inflammation.

Conclusions

Progress in biology generally occurs through a series of small advances that serve to amplify, refine or clarify an existing concept or paradigm. Changes in the underlying paradigm occur much less frequently, but when they do, they open grand new vistas on our understanding of disease. Such a paradigm shift has occurred with the recognition of programmed cell death as a process intrinsic to normal growth and development and to the host response to its environment. The scientific literature on apoptosis has grown exponentially, and the list of disease processes in which apoptosis plays a role continues to expand. As the cellular processes responsible for the expression and regulation of apoptosis become better defined, entirely new approaches to the treatment of a wide spectrum of malignant, inflammatory and degenerative diseases should become available.

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Books Received
Livres reçus

This list is an acknowledgement of books received. It does not preclude review at a later date.

Cette liste énumère les livres reçus. Elle n'en exclut pas la critique à une date ultérieure.

Advances in Surgery. Volume 30. Editor-in-Chief: John L. Cameron. 463 pp. Illust. Mosby–Year Book, Inc., St. Louis. 1996. Can$102. ISBN 0-8151-1496-6

Atlas of Adult Foot and Ankle Surgery. Lowell D. Lutter. 342 pp. Illust. Mosby–Year Book, Inc., St. Louis. 1997. Can$211. ISBN 0-8016-6280-X

Atlas of General Thoracic Surgery. Larry R. Kaiser. 221 pp. Illust Mosby–Year Book, Inc., St. Louis. 1997. Can$145. ISBN 0-8016-6380-6

Endovascular Surgery for Aortic Aneurysms. Brian Hopkinson, Waquar Yusuf, Simon Whitaker and Frank Veith. 310 pp. Illust. W.B. Saunders Company Ltd., London. 1997. Can$117. ISBN 0-7020-2148-2

Katzenstein and Askin's Surgical Pathology of Non-Neoplastic Lung Disease. Third edition. Volume 13 in the series Major Problems in Pathology. Anna Luise A. Katzenstein. 477 pp. Illust. W.B. Saunders Company, Philadelphia. 1997. Can$116. ISBN 0-7216-5755-9

Management of Perioperative Complications in Gynecology. Vicki V. Baker and Gunter Deppe. 263 pp. Illust. W.B. Saunders Company, Philadelphia. 1997. Can$109. ISBN 0-7216-5881-4

Presenting Science with Impact: Presentation Skills for Scientists, Medical Researchers & Health Care Professionals. 112 pp. Illust. Trifolium Books Inc., Toronto, 1997. Can$16.95. ISBN 1-895579-87-2

Tibial Plateau Fractures. Mason Hohl. 195 pp. Illust. W.B. Saunders Company, Philadelphia. 1997. Can$182. ISBN 0-7216-7015-6

Year Book of Neurology and Neurosurgery 1997. Edited by Walter G. Bradley and Robert H. Wilkins. 585 pp. Illust. Mosby–Year Book, Inc., St. Louis. 1997. Can$106. ISBN 0-8151-1209-2

Year Book of Surgery 1996. Editor-in-Chief: Edward M. Copeland, III. 688 pp. Illust. Mosby–Year Book, Inc., St. Louis. 1996. Can$103. ISBN 0-8151-7795-X

The Year Book of Urology 1996. Jean B. DeKernion and Stuart S. Howards. 462 pp. Illust. Mosby–Year Book, Inc., St. Louis. 1996. Can$115. ISBN 0-8151-3483-3