# Apoptosis (Programmed Cell Death) and the Resolution of Acute Inflammation

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Biology, for the clinician, has classically encompassed the study of the life and growth of cells and organisms; death has been perceived as a pathologic process to be prevented. But over the past few years, our perception of cell death has undergone a major transformation [1]. It is now recognized that cells do not have an absolute and unchangeable lifespan, but rather that cell survival can change in response to environmental stimuli. Moreover, a moment's reflection will confirm that for a host of normal biologic processes, controlled cellular death is not only inevitable, but critical to normal development. During embryogenesis, the formation of mature organs is dependent on the controlled remodeling of tissues resulting, for example, in the formation of interdigital web spaces or the canalization of the gastrointestinal tract. Deletion of autoreactive T cells during immune maturation is essential to prevent autoimmune disease; other tissues such as blood cells, and epithelial cells of the skin, or gastrointestinal tract are constantly formed and shed during life. The programmed death of cells is called *apoptosis*.

## Apoptosis and necrosis

Cells can die a pathological or physiological death [2]. *Necrosis* is the pathological form of cell death, and not the mechanism by which cells usually die. Necrosis typically occurs when the environment of the cell is unable to support cellular metabolic needs, either because of an acute deficiency of a vital substrate such as oxygen or nutrients, or because of exposure to substances that are toxic to the vital processes of the cell. Necrosis is characterized by uncontrolled changes in cellular architecture and function, leading to the release of intracellular contents into the cellular microenvironment. The necrotic cell swells, and the membrane ruptures; intracellular contents evoke an inflammatory response, leading to the phagocytosis of cellular debris.

*Apoptosis*, on the other hand, is a tightly controlled process, initiated through the expression of an endogenous cell death program, and brought about through the sequential and co-ordinated action of intracellular enzymes. In contrast to the necrotic cell, the apoptotic cell shrinks, its chromatin condenses, and the in-

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tracellular contents become organized into membrane-bound organelles known as apoptotic bodies. These apoptotic bodies are rapidly and efficiently removed by cells of the reticuloendothelial system, without evoking an inflammatory response. The process of apoptosis proceeds rapidly in vivo, and may be missed in tissue sections, although apoptotic cells are recognized occasionally as pyknotic cells.

## Mechanisms of apoptosis

Apoptosis is a highly conserved process in evolution. In fact, contemporary understanding of the cellular mechanisms of apoptosis is grounded in studies of the mechanisms of embryologic cell death in the nematode, *Caenorhabditis elegans*. During its maturation to an adult worm, *C. elegans* loses exactly 131 cells through apoptosis, leaving 959 cells [3] 2 genes, *ced-3* and *ced-4*, which stimulated apoptosis [4] and one gene, *ced-9*, which inhibited it [5]. A human homolog of *ced-9* was identified in a B-cell lymphoma and thus called bcl-2 [6]. Bcl-2 proved to be a mitochondrial protein involved in cytochrome c and calcium transport that served as an inhibitor of apoptosis in human cells [7]. It is now recognized as one of a larger family of proteins which includes proteins that both stimulate (*bax*) and inhibit (*bcl-2*, *bad*) apoptosis.

The search for a human *ced-3* homolog identified the interleukin-1β converting enzyme (ICE) as the first member of a family of intracellular proteins that are capable of inducing apoptosis [8]. To date, at least 11 members of this family have been identified [9]. All are cysteine proteases that share the common feature of cleaving their substrates at sites adjacent to aspartic acid residues. Proteins of this family have been termed *caspases* (cysteine proteases with aspartic acid targets) [10]; ICE is now more commonly referred to as caspase-1. Caspase targets within the cell include proteins ranging from DNA repair enzymes such as poly-ADP ribose polymerase to cytoskeletal proteins including actin; they also cleave pro-forms of each other, yielding active caspase enzymes.

Apoptosis proceeds spontaneously in a variety of cell types; the apoptotic program can also be induced through signals from the environment. For instance, the engagement of a cell surface receptor called Fas by its ligand, Fas ligand, leads to caspase activation and apoptosis [11]. Interactions between Fas and Fas ligand are responsible for the maintenance of immune privilege in anatomic environments such as the anterior chamber of the eye [12]. Conversely, deficiency of Fas or Fas ligand in animals [13] or humans [14] results in the development of autoimmunity. Another well known member of this family of cell surface death receptors is the receptor for the cytokine, tumour necrosis factor (TNF), whose engagement can also induce apoptosis [15, 16]. Indeed induction of apoptosis by TNF represents the mechanism of its anti-tumour activity [17].

The final consequence of initiation of the apoptotic program is nuclear fragmentation and plasma membrane changes leading to the formation of apoptotic bodies. The nuclear changes of apoptosis can be recognized through the formation of a characteristic ladder pattern on DNA gel electrophoresis, or by flow cytometry as reduced uptake of the nuclear dye, propidium iodide. Membrane changes of apoptosis include exteriorization of phosphatidyl serine, normally located on the inner aspect of the plasma membrane, detectable as binding of annexin V by flow cytometry [18].

## Apoptosis in the pathogenesis of critical illness

Alterations in the expression of apoptosis – both acceleration and inhibition of the apoptotic program – appear to play a role in the pathogenesis of critical illness.

In 1995, Teodorczyk-Injeyan et al. showed that lymphocyte apoptosis is accelerated following burn injury, and is responsible for burn-associated lymphopenia [19]. Similar conclusions have been drawn in a rat model of burn injury in which enhanced thymocyte apoptosis is associated with immune dysfunction [20]. Fas-mediated apoptosis of circulating lymphocytes is also increased following major surgery [21].

Hepatocytes express the Fas receptor and are very sensitive to its stimulation [22]. As a result, various forms of liver injury have been associated with abnormalities in apoptosis. Apoptosis is thought to play an important role in such pathological conditions as toxic and metabolic liver injury (for example, alcoholic cirrhosis), immune-mediated injury (for example, host-versus-allograft reaction in allograft rejection) and various viral liver diseases [23]. Liver failure in patients suffering from viral fulminant hepatitis has been shown to be a result of the apoptotic death of Fas expressing hepatocytes [24]. Similarly, interactions between Fas and its physiologic ligand have been implicated in the pathogenesis of acute Wilson's disease, with activation of Fas as consequence of the interaction of the hepatocyte with copper [25]. Injection of mice with anti-Fas antibody results in fulminant liver failure as a consequence of increased hepatocyte apoptosis [26]. Furthermore, accelerated apoptosis has been implicated as the mechanism of tissue injury in the liver and spleen in experimental endotoxin shock [27].

Under normal physiologic conditions, the expression of apoptosis in the adult kidney is uncommon. However, apoptosis appears to play a role in a number of pathologic states. Experimental models of partial ureteric obstruction and renal artery stenosis reveal an important relationship between restricted renal blood supply and loss of renal mass due to apoptotic cell death [28]. Diabetes and hypertension associated renal damage have also been shown to involve apoptosis, as has immune mediated glomerulonephritis [29].

The exotoxin of *Clostridium difficile*, the causative agent of pseudomembranous colitis, has been demonstrated to induce the apoptosis of intestinal epithelial cells [30]. Inhibition of apoptosis by viral proteins is a common mechanism in acute viral infections [31, 32]. In contrast, HIV infection induces lymphopenia, in part, through the effects of the virus in upregulating expression of Fas ligand on host macrophages [33].

## Delayed neutrophil apoptosis in systemic inflammation

Impaired expression of apoptosis may also contribute to the pathogenesis of critical illness. The development of a syndrome of sustained and exaggerated inflammation, popularly known as the Systemic Inflammatory Response Syndrome (SIRS) [34], is the predominant risk factor for the development of the multiple organ dysfunction syndrome (MODS), the leading cause of death in critical illness [35]. While infection is an important cause of SIRS, the syndrome can also develop following multiple trauma, burns, acute pancreatitis, major surgery, and other acute insults associated with the activation of an endogenous inflammatory response.

Through the release of proteases and toxic oxygen metabolites, neutrophils play an important role in the pathophysiology of SIRS as mediators of tissue injury and organ dysfunction [36, 37]. The lifespan of the neutrophil in vivo is short, probably not longer than 5 or 6 hours, and is terminated through the activation of a constitutively expressed cell death program that induces neutrophil apoptosis, and their removal by cells of the reticuloendothelial system [18]. Thus neutrophil-mediated inflammation is terminated through the programmed cell death of the neutrophil. Expression of apoptosis can be inhibited by a large number of inflammatory mediators of both microbial and host origin [38, 39]. The stimuli that inhibit the constitutive expression of neutrophil apoptosis are many, and include microbial products such as endotoxin and fMLP, cytokines such as IL-1β, IL-8, and GM-CSF, and the process of transmigration into an inflammatory focus, or the cross-linking of cell surface β2 integrins [40].

### *Neutrophil apoptosis and the pathogenesis of ARDS*

Although the acute respiratory distress syndrome has been described in neutropenic patients [41], a large body of clinical and experimental data supports the concept that neutrophils contribute to the evolution of acute lung injury [42]. Autopsy studies typically show massive accumulation of neutrophils in the lungs of patients with ARDS, and, at least in ARDS associated with sepsis, nonsurvivors show persistently higher numbers of neutrophils on bronchoalveolar lavage [43]. Concentrations of the PMN chemoattractant, IL-8, are increased in BAL fluid, and correlate with both neutrophil counts and increased risk of mor-

tality [44]. Neutrophils recovered from the lung of the patient with ARDS are activated, as manifested by increased expression of β2 integrins and shedding of L-selectin [45]. Moreover patients with ARDS have elevated levels of hydrogen peroxide in the exhaled gas [46]. Finally, there are at least two case reports documenting deterioration of ARDS in critically ill patients given G-CSF [47] or GM-CSF [48], cytokines that increase circulating neutrophil numbers by augmenting their release from the bone marrow, and by inhibiting apoptosis.

There is no evidence that neutrophils that have migrated into the injured lung are able to return to the blood stream, nor that lymphatic drainage provides an important disposal route [49]. Thus the removal of neutrophils from the lung in ARDS appears to require the apoptotic death of the cell [50]. Lung neutrophil apoptosis is delayed during experimental acute lung injury [51], as well as in patients with ARDS [52]. BAL fluid from patients with ARDS inhibits the apoptosis of control neutrophils, an effect that can be reversed with antibodies to G-CSF or GM-CSF [52]. Serial studies in ARDS have suggested that patients who survive ARDS following sepsis have an increase in alveolar macrophage neutrophil ratio over time [53], consistent with the removal of neutrophils by macrophages. The cytokine, IL-10, has also been shown to hasten the resolution of pulmonary inflammation by promoting the restoration of normal neutrophil apoptosis [54].

### *Neutrophil apoptosis in SIRS*

Neutrophils collected from patients following burn injury showed delayed apoptosis; the apoptotic delay can be reversed with a neutralizing antibody to GM-CSF [55]. Similar observations have been made in victims of multiple trauma: delayed neutrophil apoptosis is partially reversed by neutralization of G-CSF, but not of GM-CSF [56]. Our group has shown that patients with SIRS, and patients who have undergone major elective surgery (elective repair of an abdominal aortic aneurysm), show delayed neutrophil apoptosis (Fig. 1), in association with evidence of neutrophil activation, reflected in augmented respiratory burst activity [57] (Fig. 2). This delay is at least partially mediated by antiapoptotic factors in the plasma, as plasma from patients with SIRS is able to delay the apoptosis of normal control neutrophils.

## Conclusions

A better understanding of the mechanisms of apoptosis should lead to new approaches to treat a broad spectrum of diseases. New therapeutic strategies will emerge as the molecular mechanisms involved in the cell's program of apoptosis are defined. For example, caspase inhibitors have been shown to have a protective effect in animal models of apoptosis induced liver disease caused by en-

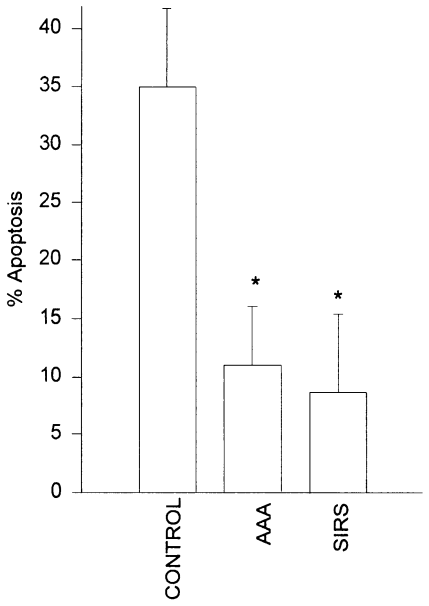


**Fig. 1.**
Expression of apoptosis is inhibited in patients with SIRS, or those who have undergone an elective repair of an abdominal aortic aneurysm. Rates of apoptosis were determined as the uptake of propidium iodide by flow cytometry. Modified from [57]

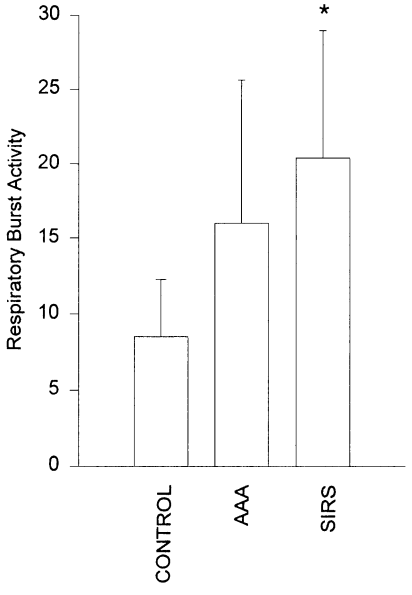


**Fig. 2.**
Concomitant with delayed apoptosis, neutrophils from patients with SIRS, or patients having undergone elective aneurysmectomy, show enhanced respiratory burst activity; data reflect log mean channel fluorescence of dihydrorhodamine 123. Modified from [57]

gagement of the Fas or TNF [58]. Other agents capable of modulating the expression of apoptosis may find a role in the treatment of SIRS in the ICU setting. For example, the normal expression of apoptosis in inflammatory neutrophils can be restored by inhibition of ICE [59], by IL-10 [54], or by inhibition of calpain [60]. Ironically, an improved understanding of the mechanisms of cell death may provide new insights into preventing mortality in the ICU.

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