Review

Science review: Apoptosis in acute lung injury

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Abstract

Apoptosis is a process of controlled cellular death whereby the activation of specific death-signaling pathways leads to deletion of cells from tissue. The importance of apoptosis resides in the fact that several steps involved in the modulation of apoptosis are susceptible to therapeutic intervention. In the present review we examine two important hypotheses that link apoptosis with the pathogenesis of acute lung injury in humans. The first of these, namely the 'neutrophilic hypothesis', suggests that during acute inflammation the cytokines granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor prolong the survival of neutrophils, and thus enhance neutrophilic inflammation. The second hypothesis, the 'epithelial hypothesis', suggests that epithelial injury in acute lung injury is associated with apoptotic death of alveolar epithelial cells triggered by soluble mediators such as soluble Fas ligand. We also review recent studies that suggest that the rate of clearance of apoptotic neutrophils may be associated with resolution of neutrophilic inflammation in the lungs, and data showing that phagocytosis of apoptotic neutrophils can induce an anti-inflammatory phenotype in activated alveolar macrophages.

Keywords adult respiratory distress syndrome, apoptosis, epithelial cells, inflammation, neutrophils

Apoptosis is a process of controlled cellular death whereby the activation of specific death-signaling pathways leads to deletion of cells from tissue. These death-signaling pathways can be activated in response to receptor–ligand interactions, environmental factors such as ultraviolet light and redox potential, and internal factors that are encoded in the genome ('programmed cell death'). Ultimately, apoptosis results in fragmentation of the DNA, a decrease in cell volume, and phagocytosis of the apoptotic cell by nearby phagocytes. Inappropriate activation or inhibition of apoptosis can lead to human disease either because 'undesired' cells develop prolonged survival or because 'desired' cells die prematurely. In addition, phagocytosis of some apoptotic cells, such as neutrophils, can induce changes in the activation phenotype of lung macrophages [1]. The importance of apoptosis resides in the fact that several steps that are involved in its modulation are susceptible to therapeutic intervention.

Two main hypotheses that link apoptosis with the pathogenesis of acute respiratory distress syndrome (ARDS) have been postulated, namely the 'neutrophilic hypothesis' and the 'epithelial hypothesis'. The former hypothesis suggests that neutrophil apoptosis plays an important role in the resolution of inflammation, and predicts that inhibition of neutrophil apoptosis or inhibition of clearance of apoptotic neutrophils is deleterious in ARDS [2,3]. The epithelial hypothesis suggests that the epithelial injury seen during ARDS is associated with apoptotic death of alveolar epithelial cells in response to soluble mediators such as soluble Fas ligand, and predicts that blockade of such inhibitors may be beneficial in preventing or treating ARDS [4,5]. These two hypotheses are not mutually exclusive, and both could play an important role in the pathogenesis of ARDS. In the present review we evaluate the evidence supporting each of these two hypotheses, with emphasis on the main modulatory steps that are candidates for therapeutic intervention.

ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; GM-CSF = granulocyte/macrophage colony-stimulating factor; IL = interleukin; SP-A = surfactant protein A.

Neutrophil apoptosis and acute lung injury

Neutrophil apoptosis affects the pathogenesis or resolution of acute lung injury by three main mechanisms. The first mechanism relates to the rate at which neutrophils become apoptotic, and how this rate is influenced by soluble mediators that are present in the inflammatory microenvironment. The second mechanism pertains to the clearance of apoptotic neutrophils by surrounding phagocytes, and how changes in this clearance rate affect the resolution of inflammation. The third mechanism is related to how phagocytosis of apoptotic neutrophils affects the activation phenotype of phagocytic cells, potentially changing it from proinflammatory to anti-inflammatory.

Rate of neutrophil apoptosis and acute lung injury

Studies in humans have shown that bronchoalveolar lavage (BAL) fluids from patients with early ARDS inhibit the rate at which neutrophils develop apoptosis *in vitro* [6]. This inhibitory effect disappears at later stages of ARDS, as inflammation resolves. The inhibitory effect of BAL fluids on neutrophil apoptosis is mediated by soluble factors, primarily the proinflammatory cytokines granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor (GM-CSF), and possibly IL-8 and IL-2 [7–10]. However, the importance of inhibition of neutrophil apoptosis in ARDS is debated because there is no clear association between the ability of BAL fluids to induce neutrophil apoptosis and the outcome of patients with ARDS, or progression to ARDS in patients who are at risk for the disease [6]. In fact, the patients who survive have higher concentrations of GM-CSF in their BAL fluids [6]. The lack of association between survival and inhibition of neutrophil apoptosis in humans does not necessarily mean that modulation of neutrophil apoptosis is irrelevant to the pathogenesis and resolution of inflammation during ARDS. This is because survival in human ARDS is affected by many factors that are difficult to control, including the presence of other diseases (e.g. diabetes, heart disease, chronic obstructive pulmonary disease) and ventilatory strategies, among other factors. Therefore, the importance of modulation of neutrophil apoptosis in the pathogenesis of acute lung injury has been studied using animal models.

Parsey and coworkers [11] measured the proportion of apoptotic neutrophils in the lungs of mice over 48 hours after endotoxemia or hemorrhage. Apoptosis was measured using the cell surface marker annexin-V in neutrophils isolated from the lung parenchyma. Immediately after hemorrhage or endotoxemia, the proportion of apoptotic neutrophils was $18.5 \pm 1.9\%$. This proportion decreased significantly 1 hour after the insult, remained low for 24 hours, and returned to baseline at 48 hours. That study confirms the human observations suggesting that neutrophil apoptosis is inhibited early in inflammation but it does not clarify the role played by neutrophil apoptosis inhibition in the pathogenesis of the injury.

This question was addressed in a subsequent study that investigated whether enhancement of neutrophil apoptosis attenuates lung injury in a murine model of ischemia/reperfusion. Sookhai and coworkers [12] demonstrated that aerosolization of opsonized killed *Escherichia coli* enhanced neutrophil apoptosis in the lungs of mice. They then showed that the mortality and the lung injury that follows ischemia/reperfusion decreased significantly when neutrophil apoptosis was enhanced by aerosolization of dead *E coli*. That study suggests that in acute lung injury enhancement of neutrophil apoptosis is beneficial to the host.

Clearance of apoptotic neutrophils and acute lung injury

The studies cited thus far focused on identifying associations between the rate at which neutrophils become apoptotic and the pathogenesis of acute lung injury. Clearance of apoptotic cells by phagocytes also plays a role in survival and persistence of inflammation during acute lung injury [13]. Macrophages and other phagocytic cells recognize apoptotic cells via a number of membrane surface molecules. One of these membrane molecules, namely CD44, appears to play an important role in the clearance of apoptotic neutrophils *in vivo* and *in vitro* [14,15]. Teder and coworkers [14] demonstrated that mice deficient in CD44 failed to clear apoptotic neutrophils in a model of bleomycin-induced lung injury. Failure to clear apoptotic neutrophils was associated with worsened inflammation and increased mortality. Adoptive transfer of normal marrow cells into the CD44-deficient mice reversed the defect in apoptotic cell clearance and improved survival. However, CD44 can increase the synthesis of chemokines such as IL-8 by enhancing clearance of the glycosaminoglycan hyaluronan [16], and it is not possible to determine whether the improvement in outcome in this model of lung injury was due to the effects of CD44 on clearance of apoptotic neutrophils or to the effect of CD44 on chemokine production.

Additional studies conducted by Hussain and coworkers [17] support the hypothesis that the rate of clearance of apoptotic neutrophils is important for the resolution phase of lung injury. Those investigators showed that the resolution of oleic-acid-induced lung injury in rats is associated with generalized apoptosis of neutrophils and with uptake of apoptotic neutrophils by alveolar macrophages, but the data did not show a definitive causal relationship. Further studies are needed to demonstrate conclusively that changes in the rate of clearance of apoptotic neutrophils can affect outcome in acute lung injury.

Phagocytosis of apoptotic neutrophils and release of cytokines by macrophages

The third mechanism whereby apoptosis of neutrophils can modify the inflammatory response is by modulating the production of proinflammatory cytokines by alveolar macrophages. Phagocytosis of apoptotic neutrophils by macrophages inhibits macrophage production of proinflammatory cytokines (i.e. IL-1β, IL-8, IL-10, GM-CSF, and tumor necrosis factor-α) and increases release of anti-inflammatory mediators (i.e. transforming growth factor-β_1, prostaglandin E_2, and platelet-activating factor) [1,18]. These findings raise

the possibility that increases in phagocytosis of apoptotic neutrophils could favor resolution of inflammation by down-regulating the inflammatory phenotype in activated alveolar macrophages.

Epithelial cell apoptosis in the pathogenesis of acute lung injury

In addition to neutrophil alveolitis, the main features of ARDS include destruction of the alveolar epithelium, with severe damage to the alveolar capillary barrier and major increases in alveolar capillary permeability. In studies investigating the morphologic changes that occur early in human ARDS, Bachofen and Weibel [19] noted that, early in the course of ARDS, type I pneumocytes exhibit decreased size and condensation of the chromatin. The alveolar epithelium of patients who die from lung injury contains cells that exhibit evidence of DNA fragmentation [20], and alveolar pneumocytes from humans with diffuse alveolar damage show upregulation of *Bax*, a *Bcl-2* analog that favors apoptosis [21]. Evidence of extensive alveolar epithelial cell apoptosis has been described in murine models of pulmonary fibrosis and lipopolysaccharide-induced lung injury [22–25]. Apoptosis of alveolar epithelial cells is detectable in mice as early as 6 hours after intratracheal administration of lipopolysaccharide [25].

The mechanisms that are responsible for epithelial cell apoptosis in acute lung injury are incompletely understood, but several lines of evidence point to the Fas/Fas ligand system [4,22,26,27]. The Fas/Fas ligand system is comprised of the cell membrane surface receptor Fas (CD95) and its natural ligand, namely Fas ligand [28]. Fas ligand exists in a membrane bound form and a soluble form, both of which are capable of inducing apoptosis of susceptible cells [4,27]. Alveolar and airway epithelial cells express Fas on their surface [29–31], and the expression of Fas in epithelial cells increases in response to inflammatory mediators such as lipopolysaccharide [22].

The soluble form of Fas ligand has been detected in several human lung diseases, including pulmonary fibrosis, bronchiolitis obliterans with organizing pneumonia, and ARDS [4,32,33]. In humans with early ARDS, soluble Fas ligand is present in the BAL fluid, and reaches higher concentrations in the lung fluids from patients who die [4]. The Fas ligand present in the lung fluids from patients with ARDS is biologically active and can induce apoptosis in normal human distal lung epithelial cells.

Several factors modulate Fas-mediated apoptosis of alveolar epithelial cells. Surfactant protein A (SP-A), the primary protein present in pulmonary surfactant, is an inhibitor of type II apoptosis *in vivo* [34,35]. This is important because, in patients with early ARDS, the concentration of SP-A is decreased in BAL fluid [36]. The lower concentration of SP-A would favor apoptosis of type II cells in these patients. Another important modulator of Fas ligand in the lungs is angiotensin II. Epithelial cells interact with angiotensin II via the angiotensin receptor subtype AT_1, and this interaction is required for Fas-mediated apoptosis of alveolar epithelial cells *in vitro* [37]. In ARDS the concentration of angiotensin-converting enzyme, which catabolizes the conversion of angiotensin I to angiotensin II, is increased in BAL fluid [38]. Therefore, in early ARDS a combination of three factors favors alveolar epithelial apoptosis: increased concentrations of soluble Fas ligand; decreased concentrations of SP-A; and increased concentrations of angiotensin-converting enzyme and angiotensin II.

Several lines of research in animals support the hypothesis that activation of the Fas/Fas ligand system is important in the pathogenesis of acute lung injury. Administration of the monoclonal antibody Jo2, which binds and activates Fas, results in alveolar epithelial cell apoptosis, neutrophilic lung inflammation, and permeability changes in mice [27]. A single administration of Jo2 is followed 6 and 24 hours later by changes in alveolar permeability and neutrophil recruitment, whereas chronic administration of Jo2 leads to the development of pulmonary fibrosis [27,33]. This phenomenon is associated with evidence of DNA fragmentation in cells of the alveolar epithelium [27]. Human recombinant Fas ligand can also induce lung injury in animals. In rabbits, human recombinant Fas ligand at low doses produces neutrophilic alveolitis and permeability changes, whereas higher doses result in hemorrhagenic lung injury [26]. Thus, activation of the Fas/Fas ligand system *in vivo* is associated with two phenomena: the first is apoptosis of the alveolar epithelium with epithelial damage and increased alveolar permeability; and the second is increasing inflammatory cytokines and neutrophil recruitment.

Conclusion

A growing body of evidence implicates apoptosis in the pathogenesis and resolution of ARDS. Studies in humans and animals suggest that neutrophil apoptosis is inhibited early in ARDS and that the lifespan of neutrophils returns to normal as inflammation resolves. Furthermore, the rate of clearance of apoptotic neutrophils may play an important role in the resolution of the inflammatory response. Apoptosis of cells of the alveolar epithelium mediated by the Fas/Fas ligand system may also be of particular importance in the development of the permeability changes that are characteristic of ARDS. Further research is necessary to identify anti-apoptotic therapeutic targets that may be useful in the treatment of human ARDS.

Competing interests

None declared.

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