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Apoptosis in Lung Transplantation

Stefan Fischer and Shaf Keshavjee

Apoptosis or *programmed cell death* is currently an area of intense clinical and experimental interest. This process plays a significant role in tissue development and homeostasis. The number of diseases in which it is becoming evident that apoptosis plays a role as a causal or protective factor is rapidly increasing. The induction of cellular apoptosis presents an exciting potential therapeutic approach in the treatment of diseases such as cancer. In clinical and experimental transplantation, it appears that apoptosis aids in the removal of cells that are damaged during the overall process of transplantation because of acute injury or immunologic processes. In the transplanted lung, a significant amount of graft cells undergo apoptosis in the very early phase following graft reperfusion. This active and silent genetic shutdown of damaged cells relates to the length of ischemic preservation. The underlying molecular mechanisms of apoptosis induction in transplanted lungs remain to be discerned. In this review, we provide an overview of apoptosis with respect to its potential regulation in the setting of lung transplantation, particularly with respect to ischemia-reperfusion injury.

Introduction

Lung transplantation has become a widely accepted standard treatment modality for end stage lung diseases, such as cystic fibrosis (CF), emphysema, primary pulmonary hypertension, idiopathic pulmonary fibrosis, and others. Since the initial success in Toronto in 1983, approximately 14,000 lung transplantations have been performed worldwide, as reported by the Registry of the International Society for Heart and Lung Transplantation in 2000.²

Ischemia Reperfusion Injury

In the very early phase after lung transplantation, severe life-threatening graft dysfunction related to ischemia-reperfusion (IR) injury occurs in up to 20% to 30% of patients, which significantly contributes to the overall 1st year mortality of approximately 25% of patients posttransplant.³ Clinically, IR injury manifests as pulmonary edema and acute respiratory distress syndrome, characterized by significant pulmonary infiltrates and severe oxygenation difficulties.⁴ Consequently, patients are subjected to prolonged mechanical ventilatory support, with or without inhaled nitric oxide (NO), ventilator-associated infections, longer stays

in the intensive care unit, and increased early postoperative mortality.⁵

Pathophysiology of IR Injury

Endothelial dysfunction is an important early phenomenon in virtually all forms of ischemia-reperfusion, including organ transplantation.⁶ The dysfunction appears to be triggered within minutes of the endothelial generation of a large burst of superoxide radicals.⁷ However, the initial dysfunction may be amplified by neutrophil-generated factors, including oxygen-derived free radicals, proinflammatory cytokines, and lipid peroxidation.⁸ Moreover, activated neutrophils migrate across the endothelium into the pericapillary space,⁹ where they cause tissue destruction by releasing oxygen free radicals, proteolytic enzymes (cathepsin G, elastase, collagenase), and peroxidase.¹⁰ Sequestration of activated neutrophils is an important step in the development of graft failure, or even multiple organ failure following transplantation.¹¹ The pathophysiology of IR injury in the lung following transplantation also involves platelet activation and tissue inflammation, followed by alveolar-capillary barrier leakage, ultimately causing interstitial and alveolar edema and

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cell injury.¹² Lipid peroxidation is the most damaging effect of free radicals in IR injury.¹³

Cell death following exposure to different stimuli, for example, heat stress, is primarily due to free radical reactions with DNA.¹⁴ The release of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and interleukin-2 (IL-2), as well as the up-regulation of their receptors (such as IL-2R),¹⁵ are involved in the complex system that mediates IR injury following pulmonary graft preservation and transplantation.

Currently, there is no reliable, reproducible histological IR injury scoring system available to measure accurately the degree of tissue or cellular injury during preservation and reperfusion in transplanted lungs. Edema, pulmonary parenchymal and airway inflammation,¹⁶ neutrophil migration, intraparenchymal hemorrhage, and cell death (apoptosis and necrosis) have all been utilized as histological markers of injury following organ transplantation.¹⁷ In general, however, such examinations of the pathological correlates of IR injury have not been particularly helpful, as they are nonspecific, insensitive, and nonpredictive.

Cell Death

Recently, increasing interest has been demonstrated in the induction of cell death in transplanted organs during ischemia and after transplantation. A basic premise of transplantation rests upon the theoretical belief that cells can endure a degree of injury and still maintain the ability to recover. Ultimately, however, if a sufficient degree of injury is inflicted upon a cell, the cell may die. There are two major ways in which this might occur—namely, *necrosis* and *apoptosis*.

Necrosis

Necrosis is a form of irreversible cell death accompanied by the loss of plasma membrane integrity and ion pump damage, leading to the loss of sodium and calcium ion balance, followed by acidosis, osmotic shock, clumping of chromatin, and nuclear disruption.¹⁸ Sodium ions move into the cell, drawing with them a volume of water to maintain osmotic equilibrium with the surrounding interstitial space, while potassium ions escape from the cell into the interstitium.¹⁹ These changes are

accompanied by activation of mitochondrial phospholipase, a precipitant loss of oxidative phosphorylation, and a drop in adenosine tri-phosphate (ATP) production, which leads to failure of synthetic and homeostatic capability.¹⁸ Calcium overload leads to mitochondrial membrane dysfunction and irreversible damage. Secondary autolysis, accompanied by lysosomal swelling, dilatation and vesiculation of the endoplasmic reticulum, and release of intracellular enzymes and proteins, and the loss of cellular compartmentalization occur. In vivo, necrosis of cells induces neutrophil migration, a significant tissue inflammatory response, and edema.²⁰

Apoptosis

An alternate form of cell death is apoptosis or *programmed cell death*. Physiologically, apoptosis is a genetically controlled mechanism used to eliminate aging, genetically damaged, or “sick” cells. Thus, as a counterpart to mitosis, apoptosis is an important regulator in development and maintenance of tissue homeostasis in multicellular organisms.²¹ Glucksmann originally described the death of cells during normal development of vertebrates and invertebrates in 1951.²² In 1965, Kerr described different types of liver cell death after portal vein branch ligation.²³ In 1972, Kerr and Searle proposed the name “apoptosis” for this phenomenon (from the Greek, $\alpha\pi\omicron\tau\omicron\varsigma$, which translates as “falling off”) and suggested it might play a role opposite to that of mitosis.

As observed by electron microscopy, apoptotic cell death is morphologically characterized by overall cellular condensation, shrinkage, and plasma membrane blebbing.²⁰ Nuclear changes are characterized by chromatin margination and nuclear condensation followed by segmentation. During this time, DNA fragmentation into 180 to 200 base pair (bp) fragments occurs. Finally, the apoptotic cell is broken into smaller membrane-bound apoptotic bodies that are usually phagocytosed by macrophages.²⁴ Using both light microscopy and electron microscopy, an important morphologic distinction from necrosis lies in the fact that apoptotic cell death lacks inflammation.²⁴ Daemen and colleagues, however, have recently described a possible association between the occurrence of apoptosis

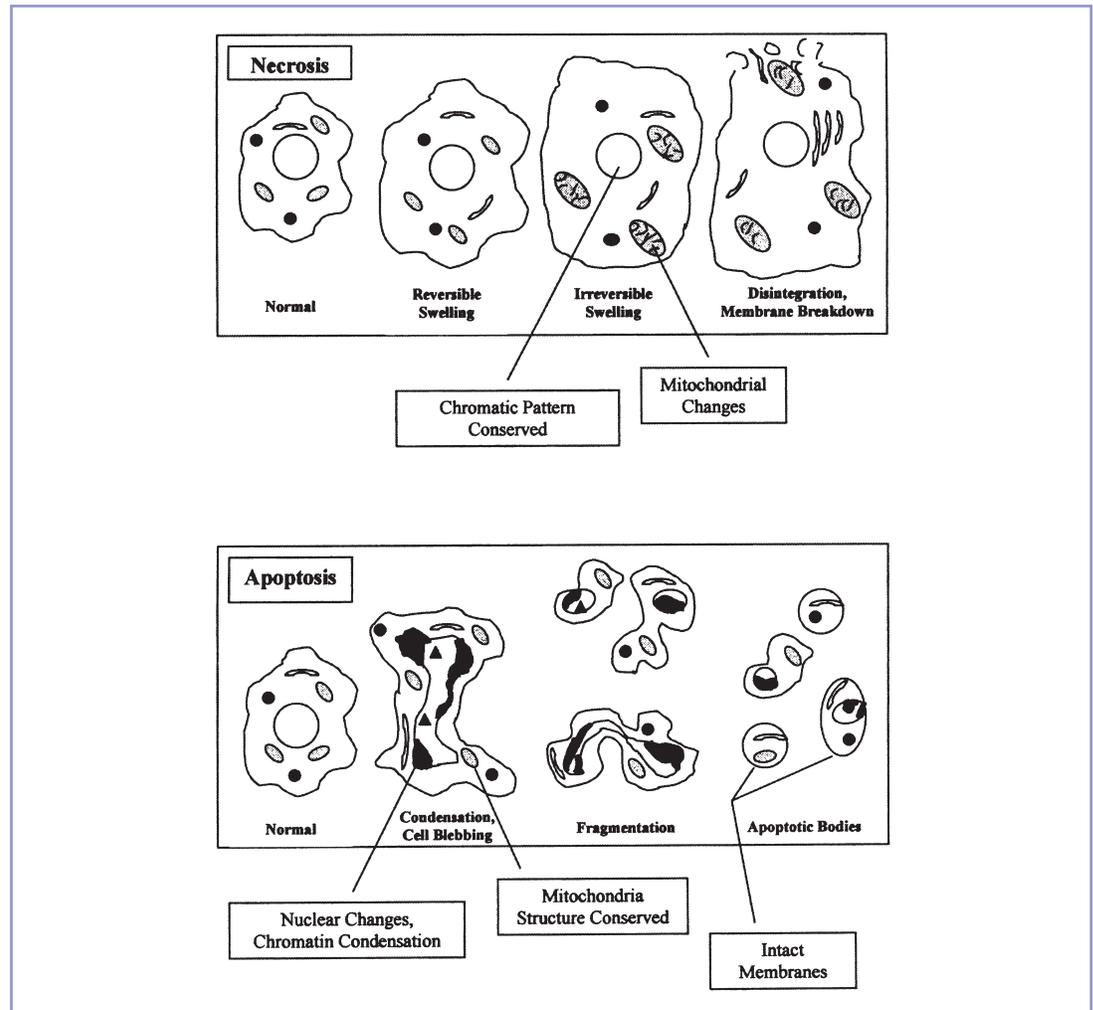


Figure 1. Morphological differences between apoptosis and necrosis.

and neutrophil influx and sequestration in reperfused kidneys.²⁵ The mechanism for this phenomenon, which suggests that apoptosis may indeed lead to secondary inflammation, is not clear. However, hypothetical saturation of the apoptotic cell removal systems (i.e., the macrophage system) by an overwhelming number of apoptotic cells, might subsequently lead to *secondary* necrosis of apoptotic cells and thus cause inflammation. Furthermore, the extent of cellular necrosis in these organs was not reported. The characteristic morphological features of the two different modes of cell death—necrosis and apoptosis—are depicted in Figure 1.

Role of Apoptosis in Disease

There are strong indications to believe that the dysregulation of apoptosis plays an important regulatory role in various disease processes, such as cancer, autoimmune diseases, and other disease states.²⁶ Increasing evidence suggests that alterations in the genetic control of cell survival and elimination are important regulators in these disease states. Of particular importance in organ transplantation, apoptosis has recently been reported in several clinical and experimental studies following IR injury to the kidney, retina, brain, heart, liver, and adrenal gland.²⁷⁻³²

Many laboratory studies have demonstrated a relationship between organ injury (including IR injury) and apoptosis induction. However, it is unknown whether a more significant injury triggers necrosis and a milder injury initiates apoptotic cell death. It has been reported that apoptosis appears in rabbit cardiomyocytes after ischemia followed by reperfusion, yet it is not detectable following ischemia alone.²⁸ These findings are of particular interest to transplantation medicine and surgery.

Presently, very little is known about the relationship between IR injury and the induction of apoptosis after human organ transplantation. Isolated reports of a descriptive nature are available regarding apoptosis in human liver, kidney, pancreas, and heart transplantation.³³⁻³⁵

Apoptosis in Human Lung Transplantation

In a clinical study designed to determine if apoptosis is present in human lung tissue before, during, and after transplantation, we collected tissue biopsies from 20 consecutive human lungs for transplantation following cold ischemic preservation (1 to 5 h), after warm ischemia time (during implantation), and 30, 60, and 120 min following graft reperfusion.³⁶ Apoptosis was detected and quantified by fluorescent *in situ* end labeling of DNA fragments (TUNEL). Almost no positive staining was detected in specimens following both cold and warm ischemic periods. Significant increases in the numbers of cells undergoing apoptosis were observed following graft reperfusion in a time-dependent manner. The mean fraction of apoptotic pulmonary cells at 30, 60, and 120 min after graft reperfusion were 16.6%, 22.1%, and 34.9% of total graft cells, respectively. As confirmed by electron microscopy, the majority of apoptotic cells were alveolar type-II pneumocytes.³⁶ We concluded that apoptosis appears to be a significant type of cell death following human lung transplantation, which may contribute to lung dysfunction during the early phase of graft reperfusion. This knowledge is of importance for potential future development of interventions to prevent lung dysfunction after transplantation, perhaps by using antiapoptotic strategies to limit programmed cell death.

Cell Death in Experimental Lung Transplantation

Following organ transplantation, the mode of cell death appears to be related to the ischemic time and the process of reperfusion. Recently, we developed a triple-staining technique to quantify apoptosis and necrosis in the same specimens of lung tissue,³⁷ a technique that has been validated by other groups working on cell culture models.³⁸ We used this method (which quantifies the degree of apoptosis versus necrosis) to determine the time course of each type of cell death during lung preservation, transplantation, and reperfusion. We determined the impact of the specific types of cell death on posttransplant pulmonary function.³⁷ We noted that apoptosis is the predominant type of cell death in transplanted lungs following moderate periods of cold storage (6 and 12 h), with 28% of cells being apoptotic after reperfusion for both of these times. The amount of dead cells in these lungs after hypothermic storage (prior to reperfusion) was less than 2%. On the other hand, necrosis was the predominant type of cell death after long-term storage prior to reperfusion (18 and 24 h). In these lungs, necrotic cell death was already evident after cold storage in approximately 10% and 24% of graft cells, respectively. The percentages of necrotic cells in these lungs after transplantation were approximately 21% and 29%, respectively, whereas the amount of apoptotic cells remained low, at levels of less than 2%. Table 1 illustrates the amounts of apoptosis and necrosis in lungs at various states of the lung transplantation process.

Furthermore, the amount of necrotic cells in the lungs after transplantation correlated with the degree of organ dysfunction 2 h after reperfusion.

Mechanisms and Signaling Pathways

Apoptosis in organ transplantation is a relatively young field of investigation, and until now there has been little in the literature that focuses on the examination of underlying signaling pathways of apoptosis induction in transplanted lungs. Thus, at present one can only extrapolate from what investigators have reported in apoptosis-related research on topics other than lung transplantation. Vaux and Strasser have recommended dividing the

Table 1 | CELL DEATH IN LUNGS AFTER DIFFERENT PERIODS OF ISCHEMIC PRESERVATION AND AFTER SAME ISCHEMIC PERIODS FOLLOWED BY TRANSPLANTATION AND 2 H OF GRAFT REPERFUSION

	APOPTOSIS	NECROSIS
Short cold ischemia (20 min)	0	0
Warm ischemia (60 min)	0	0
Reperfusion after cold ischemia (20 min)	0	0
Intermediate cold ischemia (6 and 12 h)	0	0
Reperfusion after intermediate cold ischemia (6 and 12 h)	+++	0
Extended cold ischemia	0	++/+++
Reperfusion after extended cold ischemia (18 and 24 h)	0	+++

process of physiologic cell death (apoptosis) into 4 different phases for improved understanding:³⁹

1. apoptotic stimulus;
2. signal detection and transduction;
3. effector phase/caspase activation; and
4. postmortem phase/cellular degradation.

The nematode *Caenorhabditis elegans* has provided an excellent model system in which the stages of physiological cell death can be observed. During development, *C. elegans* has 1,070 cells of which 131 must die. Mutants in which cell death is abnormal have provided invaluable insight into the “death” genes that implement it.

At present, it is not clear what stimulus induces apoptosis in transplanted lungs. It is hypothesized that certain presuppositions have to be fulfilled for a cell to initiate its suicidal genetic program and undergo apoptosis. First, there must be a specific stimulus that initiates cell death. In organ transplantation, this could be an endogenous trigger such as cellular or DNA damage during ischemia or from preservation. It could also be an exogenous stimulus, such as proinflammatory cytokines, that initiates apoptosis through specific cell surface death receptors. For instance, TNF- α , a very potent apoptosis inducer, is up-regulated within 30 min following lung transplantation.⁴⁰ Second, even though the cell is injured, it must still be able to initiate the genetic program to undergo apoptosis, which is an energy-requiring process. Therefore, the cell must contain at least some ATP resources. This could possibly explain why transplanted lungs in our experimental study showed high amounts of apoptotic cells after short ischemia and of necrosis after long-term preservation. In lungs after short is-

chemic times, almost no dead cells were seen. However, it is still unknown how many cells were injured (but did not die) from ischemia before transplantation. Furthermore, it is unknown how many of these injured cells recovered after transplantation and how many underwent any type of cell death. Following lung transplantation, approximately 30% of graft cells were obviously damaged but not damaged enough to start the apoptotic program.^{36,37} In lungs after long ischemic times, 10% to 30% of graft cells were already necrotic before the lung was transplanted, suggesting that the stimulus was too strong (lethal) for the cells to undergo apoptosis. These cells died randomly by necrosis, which was worse after transplantation and reperfusion of these lungs.³⁷ We hypothesize that the high amounts of cellular necrosis in lung grafts after long-term preservation cause tissue inflammation. This inflammation is due to the release of intracellular proteins into the surrounding tissue, which further increases cellular necrosis. These novel observations indicate that the mode of cell death that occurs in transplanted lungs is dependent on the prior ischemic injury, plus the insult due to graft reperfusion following lung transplantation.

Cell Death Receptors

Various characterized apoptotic signaling pathways involve the binding of proteins, such as TNF- α , Fas-L, and TNF-related apoptosis-inducing ligand (TRAIL/Apo-3L), to their cell surface receptors TNFR1, Fas, and death receptor (DR) series.⁴¹ TNF-receptors (TNFR) bind 2 related TNF molecules: TNF- α and TNF- β . TNF- α is one of the principal mediators of inflammation in lung transplantation, and interaction between TNF and TNFRs produces a range of effects, which are depend-

ent on the nature of the target cell. The respective role of the two TNFR chains in triggering cell death, however, is disputed. Some studies suggest that TNFRp55 is the principal mediator,⁴² whereas others contend that p70 may be equally effective.⁴³ Because several of the commonly used target cells constitutively express one or both TNFRs, it has been difficult to assess the effect of each receptor chain independently. The possibility of direct or indirect cooperation between the two polypeptides has not been excluded in the literature.

Fas (Apo-1/CD95) antigen is a 43-kD cell surface glycoprotein that is constitutively expressed in a variety of normal tissues and tumor cell lines while being inducible in others.⁴⁴ Administration of anti-Fas mAb *in vitro*⁴⁴ and *in vivo*⁴⁵ triggers apoptosis of Fas-expressing cells, suggesting that Fas may play a critical role in the regulation of cell death in a broad range of tissues. Mutations that inactivate Fas have been shown to be associated with the lymphoproliferative disorder of *lpr/lpr* mice, and Fas has recently been implicated in cytotoxic T lymphocyte-mediated target cell death.⁴⁶

Although Fas and TNFRs are currently grouped together as the principal cell death receptors, it is unclear whether they trigger the death signal along the same or distinct pathways, whether the potency of each receptor in any given cell is comparable, and whether their function is subject to similar or distinct regulatory circuits. Some studies indicate that different biochemical pathways are activated by Fas and TNFR-1,⁴⁷ whereas others demonstrate a cooperation of both receptors.⁴⁸ However, besides the caspase 9-involving intracellular apoptosis activation due to drugs, toxins, and radiation, as mentioned above, there are 2 major pathways that play a role in initiating and controlling apoptosis through these receptors and their death domains, which involves caspase 8 activation. After binding of peptides such as TNF- α and Fas-ligand (Fas-L), their receptors oligomerize and recruit the adapter proteins FADD (Fas-associated death domain) and TRADD (TNF-receptor associate death domain) to form death-inducing signaling complexes (DISC).⁴⁹ This activates procaspase 8, which further activates the initiator caspase 8 (Figure 2). Caspase 8 activates downstream the effector caspases (e.g., caspase 3).⁵⁰ The TNF- α /TNFR-1 complex can also elicit an anti-apoptotic response by re-

cruiting TRAF-2 (TNF receptor associated factor 2), which results in NF- κ B-mediated up-regulation of antiapoptotic genes through activation of the stress-activated protein kinase (SAPK)/jun kinase (JNK).⁵¹

If the TNF pathway indeed regulates apoptosis in transplanted lungs in the very early stage following graft reperfusion, then the transfection of lungs with anti-inflammatory cytokines may reduce the inflammatory tissue reaction, which is worse the more cells undergo necrosis from the release of intracellular proteins by necrotic cells.²⁰ In fact, Itano and colleagues have recently shown that adenoviral-mediated transfection of rat lungs with IL-10 leads to a significant reduction in IR injury and improved graft function.⁵² This is consistent with what we have found in a similar study. In our study, we observed a decrease of TNF- α and IFN- γ levels in lung tissue, as well as in recipient plasma 2 h after transplantation of lungs that were transtracheally hIL-10-transfected using adenovirus Ad5 in the living donor 24 h prior to graft retrieval. Interestingly, in this study, we observed an increase in apoptotic cells in transplanted grafts after 24 h of cold preservation plus 2 h of reperfusion, and a significant decrease of necrotic cells. We concluded that the increased early expression of the anti-inflammatory protein IL-10 either preserved the cells' ability to undergo apoptosis even after prolonged ischemia or reduced the tremendous inflammatory reaction in lungs after such a long preservation time, which, as mentioned above, usually leads to cellular necrosis rather than apoptosis.

Conclusion

Several laboratory studies have shown a relationship between organ injury, including IR injury, and the induction of apoptosis.⁵³⁻⁵⁵ Cell death, especially apoptosis, is a tightly regulated process that involves numerous initiators as well as specific transduction and signaling pathways.

We and others have shown that there are two types of cell death that can occur in transplanted lungs: apoptosis and necrosis. Their occurrence is mainly dependent on the length of ischemia before transplantation. Apoptosis appears not to be initiated during cold ischemic preservation at 4 °C. That might be explained by the fact that apoptosis is an energy-requiring process, which is suppressed by

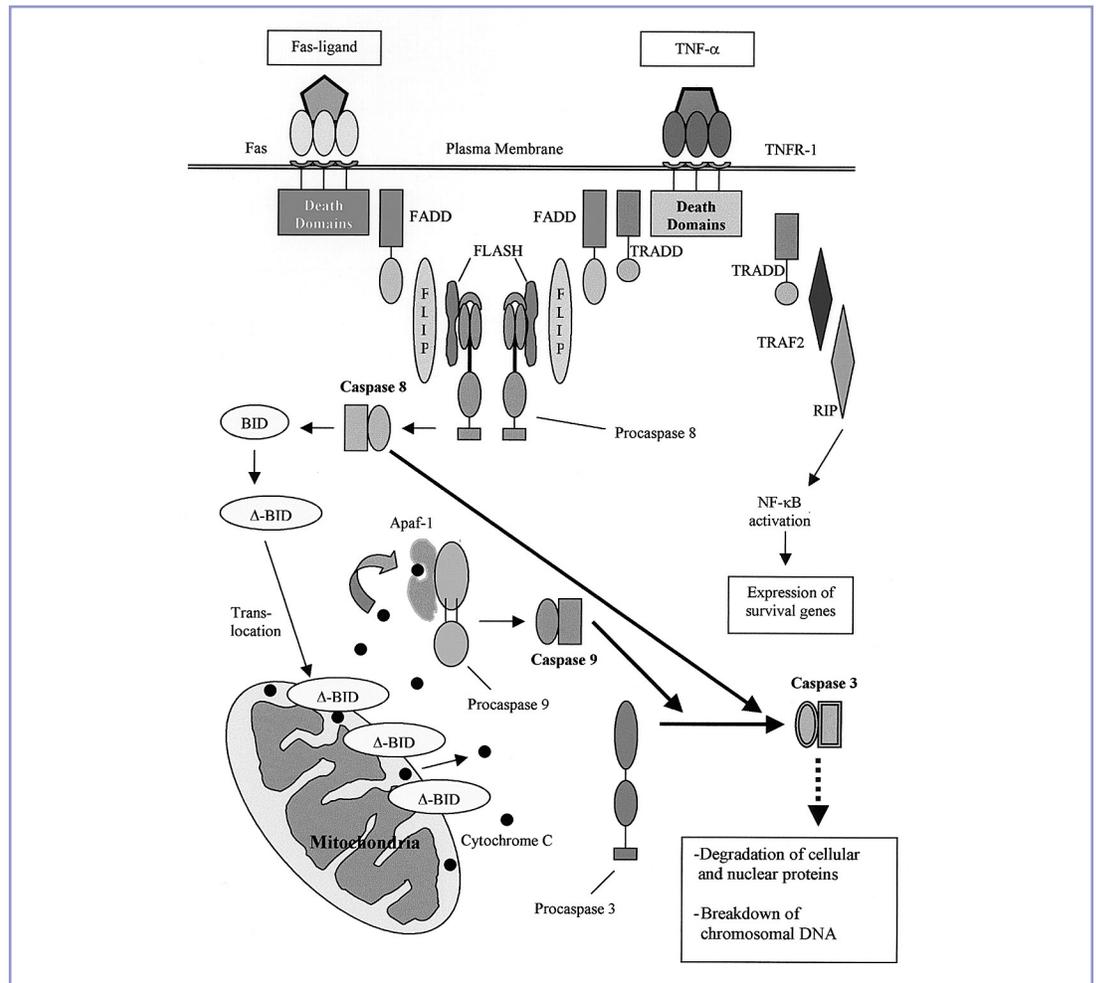


Figure 2. The two major pathways that mainly use the initiator caspase 8 to trigger effector caspases, which are initiated by the tumor necrosis factor receptor-1 (TNFR-1) or Fas.

hypothermia. However, a mild cellular injury during this phase of the lung transplantation process cannot be excluded. During warm ischemia, apoptosis levels in lung grafts following short periods of preservation remain at low levels but increase after reperfusion within the first 2 h to approximately 30%.³⁶ After long-term preservation, lungs show already high levels of necrotic cell death, which increase after reperfusion, suggesting a cellular injury due to ischemia that is beyond the ischemic limit that graft cells can survive.³⁷ From our experimental study, we would set the cutoff point of tolerable ischemic time for lung grafts at approximately 15 h. This is consistent with our observation of satisfac-

tory lung function following 12 h hypothermic preservation in our clinical lung transplant program. Yet, this fact also indicates the limitation of current techniques for lung preservation, which must be further modified to make prolonged lung preservation feasible. We recently described decreased cell death and improved function in rat lungs that were stored in a raffinose-modified low potassium dextran glucose preservation solution for 24 h.^{56,57} These and other studies suggest cell death to be an important and unique marker in lung preservation- and transplantation-related research. The most important clinical endpoint in the early phase following lung transplantation is the graft's

ability to oxygenate blood measured by blood gas analysis (arterial PaO₂ levels). We have shown a correlation between posttransplant lung function and the predominant type of cell death.³⁷ This further supports the importance of cell death in clinical and experimental lung transplantation, because it opens new avenues to improve the outcome of lung transplantation by developing strategies to prevent or modify cell death. Useful approaches might be direct caspase inhibition, transfection of anti-inflammatory genes, or modifications of current preservation strategies by cell-stabilizing agents, such as the trisaccharide raffinose.

From the limited evidence on cell death in the lung, we believe that cell death is more a result of incidences occurring at earlier time points in the lung transplantation process rather than a mechanism itself. Obviously, pulmonary graft cells become increasingly injured the longer the lung is preserved before transplantation. Thus, the "real" insult that causes lung dysfunction and the injurious trigger that later will lead to the initiation of cell death after transplantation occurs during ischemia. Consequently, the most useful approaches to improving the outcome following lung transplantation will be directed toward events before transplantation, possibly even when the graft is still in the donor individual.

Another very important question is how the transplanted lung recovers from such a high cellular loss following transplantation. Although a mechanistic relationship between IR injury, or early graft dysfunction, and chronic graft dysfunction has yet to be elucidated, abnormal healing processes and the development of a pro-fibrotic environment after posttransplant IR injury may be a possible link to obliterative bronchiolitis (OB), which is the histological manifestation of end-stage (and irreversible) chronic graft failure in transplanted lungs. Recently, Crocetti and colleagues reported on the relationship between early and late pulmonary graft dysfunction, comparing the degree of postreperfusion pulmonary edema in x-rays obtained from human lung transplant recipients and the occurrence of OB.⁵⁸ The role of apoptosis in chronic lung dysfunction after transplantation is now being investigated by a small number of research groups. However, the limited evidence that

has been achieved thus far suggests that apoptosis is the predominant mode of cell death during active OB processes in the transplanted lung.⁵⁹ Once again, however, the important question is whether apoptosis is a destructive mechanism of cell elimination initiated by immunologic processes or whether it is the response of the host to eliminate rejected graft cells to prevent cellular necrosis and damage of other nonrejected cells. This is unclear and should be investigated in future studies.

The finding that apoptosis is induced in the lung transplantation setting, with significantly elevated levels after graft reperfusion, is an intriguing observation. Clearly, further investigation is required to define the implications of apoptosis in lung transplantation. This type of cell death may indeed represent a marker of cell injury resulting from ischemic preservation, and the oxidative stress of reperfusion, heralding a poorer posttransplantation prognosis. Alternatively, apoptosis may represent a host-protective form of selective cell death, inevitable, to a certain degree, following the stresses imposed by the transplantation process. In a sense, *controlled* cellular senescence may be favored over the *destructive* and inflammatory cellular death seen in necrosis, which results in the local and systemic release of injurious and noxious intracellular components, and pro-inflammatory cytokines such as TNF- α . The idea of apoptosis being a protective mechanism in transplanted lungs following reperfusion is further supported by the observation that apoptosis can be induced with high levels of nitric oxide and that high levels of nitric oxide are clinically used to improve graft function in lungs with severe IR injury. This concept, however, has yet to be proven, but an ongoing randomized clinical trial at our institution is focusing on the role of nitric oxide in transplanted lung IR injury. Furthermore, because apoptosis is a genetically controlled, programmed cell death, it might indeed be possible to modify this type of cell death in transplanted organs with interventions, for example, gene therapy. Necrotic cell death may be more difficult to influence directly, but it's likely that this can be done indirectly by improvements in lung preservation.

Apoptosis could also be viewed as an adaptive mechanism, which aids the body in its response to a significant injury. This view of the process

hypothesizes that a high index of apoptosis in a transplanted organ is indicative of that organ's ability to eliminate injured cells and avoid the more destructive process of necrosis. This theory views apoptotic cell death *not* negatively as a loss of organ tissue and function, but positively as an important part of the process of tissue healing from reperfusion injury. This is similar to the field of fetal organogenesis where it has been demonstrated that apoptosis is an important aspect of the remodeling of an effective pulmonary alveolar-capillary interface in fetal lungs during mid to late gestation.⁶⁰

REFERENCES

- Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. *N Engl J Med* 1986;314(18):1140-5.
- Hosenpud JD, Bennett LE, Keck BM, et al. The registry of the International Society for Heart and Lung Transplantation: sixteenth official report—1999. *J Heart Lung Transplant* 1999;18(7):611-26.
- Hosenpud JD, Bennett LE, Keck BM, et al. The registry of the International Society for Heart and Lung Transplantation: seventeenth official report—2000. *J Heart Lung Transplant* 2000;19(10):909-31.
- Kundu S, Herman SJ, Winton TL. Reperfusion edema after lung transplantation: radiographic manifestations. *Radiology* 1998;206(1):75-80.
- McCurry KR, Keenan RJ. Controlling perioperative morbidity and mortality after lung transplantation for pulmonary hypertension. *Semin Thorac Cardiovasc Surg* 1998;10(2):139-43.
- Lefer AM. Endothelial dysfunction as a trigger for ischemia-reperfusion injury. *J Thromb Thrombolysis* 1997;4(1):63-5.
- Tsao PS, Aoki N, Lefer DJ, et al. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation* 1990;82(4):1402-12.
- Chen HM, Chen JC, Shyr MH, et al. Neutrophil elastase inhibitor (ONO-5046) attenuates reperfusion-induced hepatic microcirculatory derangement, energy depletion and lipid peroxidation in rats. *Shock* 1999;12(6):462-7.
- Schmid RA, Yamashita M, Ando K, et al. Lidocaine reduces reperfusion injury and neutrophil migration in canine lung allografts. *Ann Thorac Surg* 1996;61(3):949-55.
- Windsor AC, Mullen PG, Fowler AA, et al. Role of the neutrophil in adult respiratory distress syndrome. *Br J Surg* 1993;80(1):10-17.
- Deitch EA. Multiple organ failure. Pathophysiology and potential future therapy. *Ann Surg* 1992;216(2):117-34.
- Sakuma T, Takahashi K, Ohya N, et al. Ischemia-reperfusion lung injury in rabbits: mechanisms of injury and protection. *Am J Physiol* 1999;276(1 Pt 1):L137-45.
- Sakamaki F, Hoffmann H, Muller C, et al. Reduced lipid peroxidation and ischemia-reperfusion injury after lung transplantation using low-potassium dextran solution for lung preservation. *Am J Respir Crit Care Med* 1997;156(4 Pt 1):1073-81.
- Ikeda H, Suzuki Y, Suzuki M, et al. Apoptosis is a major mode of cell death caused by ischaemia and ischaemia/reperfusion injury to the rat intestinal epithelium. *Gut* 1998;42(4):530-7.
- Lawrence EC, Holland VA, Young JB, et al. Dynamic changes in soluble interleukin-2 receptor levels after lung or heart-lung transplantation. *Am Rev Respir Dis* 1989;140(3):789-96.
- Yousem SA, Paradis IL, Dauber JA, et al. Large airway inflammation in heart-lung transplant recipients - its significance and prognostic implications. *Transplantation* 1990;49(3):654-6.
- Iwanami K, Takeyoshi I, Ohwada S, et al. Comparative study of intramucosal pH in histologic damage from small bowel ischemia-reperfusion injury. *Transplant Proc* 1998;30(7):3467-8.
- Grace PA. Ischaemia-reperfusion injury. *Br J Surg* 1994;81(5):637-47.
- Chaudry IH, Clemens MG, Baue AE. Alterations in cell function with ischemia and shock and their correction. *Arch Surg* 1981;116(10):1309-17.
- Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 1995;146(1):3-15.
- King KL, Cidlowski JA. Cell cycle regulation and apoptosis. *Annu Rev Physiol* 1998;60:601-17.
- Glucksmann A. Cell death in normal vertebrates ontogeny. *Biol Rev* 1951;26:59-86.
- Kerr JFR. A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. *J Pathol Bacteriol* 1965;90:419-43.
- Gerschenson LE, Rotello RJ. Apoptosis: a different type of cell death. *Faseb J* 1992;6(7):2450-5.
- Daemen MA, van't Veer C, Denecker G, et al. Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. *J Clin Invest* 1999;104(5):541-9.
- Saikumar P, Dong Z, Mikhailov V, et al. Apoptosis: definition, mechanisms, and relevance to disease. *Am J Med* 1999;107(5):489-506.
- Li Y, Chopp M, Jiang N, et al. Induction of DNA fragmentation after 10 to 120 minutes of focal cerebral ischemia in rats. *Stroke* 1995;26(7):1252-7; discussion 1257-8.
- Gottlieb RA, Burlinson KO, Kloner RA, et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 1994;94(4):1621-8.
- Buchi ER. Cell death in the rat retina after a pressure-induced ischaemia-reperfusion insult: an electron microscopic study. I. Ganglion cell layer and inner nuclear layer. *Exp Eye Res* 1992;55(4):605-13.
- Schumer M, Colomel MC, Sawczuk IS, et al. Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. *Am J Pathol* 1992;140(4):831-8.
- Sasaki H, Matsuno T, Nakagawa K, et al. Induction of apoptosis during the early phase of reperfusion after rat liver ischemia. *Acta Med Okayama* 1997;51(6):305-12.
- Didenko VV, Wang X, Yang L, et al. Expression of p21(WAF1/CIP1/SD1) and p53 in apoptotic cells in the adrenal cortex and induction by ischemia/reperfusion injury. *J Clin Invest* 1996;97(7):1723-31.
- Borghesi-Scoazec G, Scoazec JY, Durand F, et al. Apoptosis after ischemia-reperfusion in human liver allografts. *Liver Transpl Surg* 1997;3(4):407-15.
- Boonstra JG, Wever PC, Latereveer JC, et al. Apoptosis of acinar cells in pancreas allograft rejection. *Transplantation* 1997;64(8):1211-3.
- Shaddy RE. Apoptosis in heart transplantation. *Coron Artery Dis* 1997;8(10):617-21.
- Fischer S, Cassivi SD, Xavier AM, et al. Cell death in human lung transplantation: apoptosis induction in human lungs during ischemia and after transplantation. *Ann Surg* 2000;231(3):424-31.
- Fischer S, MacLean AA, Liu M, et al. Dynamic changes in apoptotic and necrotic cell death correlate with severity of ischemia-reperfusion injury in lung transplantation. *Am J Respir Crit Care Med* 2000;162(5):1932-39.
- Walsh GM, Dewson G, Wardlaw AJ, et al. A comparative study of different methods for the assessment of apoptosis and necrosis in human eosinophils. *J Immunol Methods* 1998;217(1-2):153-6.
- Vaux DL, Strasser A. The molecular biology of apoptosis. *Proc Natl Acad Sci U S A* 1996;93(6):2239-44.
- Eppinger MJ, Deeb GM, Bolling SF, et al. Mediators of ischemia-reperfusion injury of rat lung. *Am J Pathol* 1997;150(5):1773-84.
- Magnusson C, Vaux DL. Signalling by CD95 and TNF receptors: not only life and death. *Immunol Cell Biol* 1999;77(1):41-6.
- Tartaglia LA, Rothe M, Hu YF, et al. Tumor necrosis factor's cytotoxic activity is signaled by the p55 TNF receptor. *Cell* 1993;73(2):213-6.
- Heller RA, Song K, Fan N, et al. The p70 tumor necrosis factor receptor mediates cytotoxicity. *Cell* 1992;70(1):47-56.

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44. Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 1991;66(2):233-43.
45. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al. Lethal effect of the anti-Fas antibody in mice [published erratum appears in *Nature* 1993 Oct 7;365(6446):568]. *Nature* 1993;364(6440):806-9.
46. Rouvier E, Luciani MF, Golstein P. Fas involvement in Ca(2+)-independent T cell-mediated cytotoxicity. *J Exp Med* 1993;177(1):195-200.
47. Thomas WD, Hersey P. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. *J Immunol* 1998;161(5):2195-200.
48. Porn-Ares MI, Chow SC, Slotte JP, et al. Induction of apoptosis and potentiation of TNF- and Fas-mediated apoptosis in U937 cells by the xanthogenate compound D609. *Exp Cell Res* 1997;235(1):48-54.
49. Medema JP, Scaffidi C, Kischkel FC, et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *Embo J* 1997;16(10):2794-804.
50. Sata M, Walsh K. TNF-alpha regulation of Fas ligand expression on the vascular endothelium modulates leukocyte extravasation. *Nat Med* 1998;4(4):415-20.
51. Natoli G, Costanzo A, Ianni A, et al. Activation of SAPK/JNK by TNF receptor 1 through a noncytotoxic TRAF2-dependent pathway. *Science* 1997;275(5297):200-3.
52. Itano H, Zhang W, Ritter JH, et al. Adenovirus-mediated gene transfer of human interleukin 10 ameliorates reperfusion injury of rat lung isografts. *J Thorac Cardiovasc Surg* 2000;120(5):947-56.
53. Gao W, Bentley RC, Madden JF, et al. Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. *Hepatology* 1998;27(6):1652-60.
54. DeMeester SL, Buchman TG, Qiu Y, et al. Heat shock induces IkappaB-alpha and prevents stress-induced endothelial cell apoptosis. *Arch Surg* 1997;132(12):1283-7; discussion 1287-81.
55. Clemens JA, Stephenson DT, Dixon EP, et al. Global cerebral ischemia activates nuclear factor-kappa B prior to evidence of DNA fragmentation. *Brain Res Mol Brain Res* 1997;48(2):187-96.
56. Fischer S, Hopkinson D, Liu M, et al. Raffinose improves lung preservation for 24 hours in low potassium dextran glucose solution: a histological and ultrastructural analysis. *Ann Thorac Surg* 2000;71(4):1140-5.
57. Fischer S, Hopkinson D, Liu M, et al. Raffinose improves the function of rat pulmonary grafts stored for 24 hours in low potassium dextran solution. *J Thorac Cardiovasc Surg* 2000;119(3):488-92.
58. Crocetti JG, O'Brien OB, Furukawa S, et al. The impact of reperfusion injury on the long term development of obliterative bronchiolitis (OB) in lung transplantation. *Am J Respir Crit Care Med* 2000;161(3):A507 (Abstract).
59. Hansen PR, Holm AM, Svendsen UG, et al. Apoptosis in acute pulmonary allograft rejection and cytomegalovirus infection. *APMIS* 1999;107(5):529-33.
60. Scavo LM, Ertsey R, Chapin CJ, et al. Apoptosis in the development of rat and human fetal lungs. *Am J Respir Cell Mol Biol* 1998;18(1):21-318.