

## Review

# Leukocyte Depletion as a Mechanism for Reducing Neutrophil-Mediated Ischemic-Reperfusion Injury during Transplantation

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**Abstract:** Patients undergoing transplantation are at high risk for leukocyte-mediated morbidity because of activated neutrophils and oxygen free radicals. This type of injury is most prominent during the reperfusion stage of transplantation. When tissue becomes ischemic, normal oxidation is altered (2). As oxygen is reintroduced to the system, oxygen free radical formation occurs via the oxidation of hypoxanthine by xanthine oxidase, causing destruction of the endothelium, increased permeability, and decreased organ function (2). In addition, neutrophils that may have already been activated by contact activation from the cardiopulmonary bypass circuit, accumulate in the ischemic organ at reperfusion (9,10). Activated neutrophils then release oxygen metabolites and proteolytic enzymes, which further destroy the

integrity of the vascular endothelium (11). This insult can cause edema, capillary plugging, and poor graft function (12). Recent attempts have been made to decrease the mediators of ischemic-reperfusion injury. Perhaps the most advantageous of these attempts is the removal of leukocytes during reperfusion. This has been successfully achieved using leukocyte-depleting filters before exposing the organ to systemic blood flow (14). This article is a review of ischemic-reperfusion injury and the use of leukocyte depletion during reperfusion of transplanted organs. **Keywords:** leukocyte depletion, transplantation, ischemic-reperfusion injury, reperfusion, cardiopulmonary bypass. *JECT. 2003;35:48-52*

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Recent efforts have focused on finding the pathways and mediators involved in ischemic-reperfusion injury. It is currently believed that a complex array of mechanisms are involved in this phenomenon, including neutrophil activation and oxygen free radicals (1).

Ischemia can be defined as the inability to maintain a steady-state oxidative metabolism (1). As a result, anaerobic metabolism is initiated, resulting in the accumulation of tissue metabolites, such as carbon dioxide, lactate, and hydrogen ions. Ischemia can occur anytime the heart endures severe hypotension, such as a myocardial infarction, ventricular fibrillation, or cardiogenic shock (1). However, during cardiac surgery, we most commonly think of isch-

emia as the time of cross clamp to the time of reperfusion or cardioplegia delivery. The time of ischemia can be dangerously lengthened during organ transplantation.

During surgery, reperfusion of an ischemic organ is generally completed in a controlled fashion, such as the infusion of cardioplegia solution, reperfusate solution, or cross clamp removal. The use of a reperfusion solution is advantageous because components in the solution may help attenuate variables that can cause reperfusion injury.

The pathophysiology of reperfusion injury involves three primary mechanisms:

1. Oxygen free radicals
2. Increased intracellular calcium concentrations
3. Neutrophil accumulation (1,2).

When tissue becomes ischemic, normal oxidation of hypoxanthine is altered. Under normal conditions, hypoxanthine is converted to xanthine-by-xanthine dehydroge-

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nase. However, when oxygen is unavailable, xanthine dehydrogenase readily converts to xanthine oxidase. This causes an excessive accumulation of xanthine oxidase and hypoxanthine in the tissues. This, in itself, is not problematic; however, at the time of reperfusion, oxygen is reintroduced into the system, which rapidly catalyzes the reaction of hypoxanthine to xanthine and produces superoxide anion, (an oxygen free radical) as a bi-product via xanthine oxidase (Figure 1) (3). Oxygen free radicals are highly reactive to amino acids, sugars, phospholipids, and DNA (1). The free radicals eat away at lipids within the cell membrane to increase permeability and, consequently, decrease pulmonary and cardiac function (4). Oxygen free radicals may also impair the release of nitric oxide from coronary arteries, which may produce vasospasm and thrombosis (Nitric oxide inhibits thrombus formation by inhibiting platelet and neutrophil adhesion to the endothelium [5]) (6).

Second, the concentration of intracellular calcium increases because of uncontrolled calcium influx from extracellular spaces as well as release from sarcoplasmic reticulum via L-type calcium channels (7). Calcium influx is often a consequence of oxygen free radical release at the time of reperfusion (3). This may lead to activation of plasma membrane phospholipase A-2 and arachidonic acid formation (8). Calcium influx can also cause the depletion of high-energy phosphate stores, catalytic enzyme activation, and changes in the excitation-contraction coupling of the myocyte, which can lead to stone heart (1).

The third culprit in reperfusion injury is the accumulation of activated neutrophils. Neutrophils may already be activated by contact activation via the expression of C3b on circuit tubing or they may become activated by complement fragments, such as C3a and C5a anaphylatoxins, IL-8, platelet activating factor, and leukotriene B-4, or arachidonic acid (9,10). When the neutrophil is triggered, oxidase is generated and releases oxygen metabolites (11). Proteolytic enzymes are synthesized and released, enabling the cell to become more adhesive to the endothelium (10). Adhesion molecules assisting in the interaction between the activated neutrophil and vascular endothe-

lium include P-selectin, L-selectin, E-selectin, B-2 integrins, CD11b/CD18, and immunoglobulins (8). Adhesion leads to edema and increased microvascular permeability to protein. Edema can cause capillary plugging, thus impairing blood flow, resulting in the no-reflow phenomenon (Figure 2) (1-3,12).

There have been several mechanisms implemented by cardiac and transplant teams to decrease ischemic reperfusion injury. The most commonly practiced technique is a cardioplegia or reperfusion solution used to flush the organ before it is exposed to whole blood. Components of this solution may include buffers, oncotic agents, oxygen free radical scavengers, such nutrients as glutamate aspartate, and calcium channel blockers (1,13). All components help to ameliorate the precursors, which cause reperfusion injury.

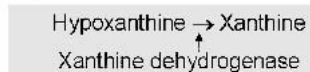
A more recent attempt to combat reperfusion injury is the depletion of leukocytes in the reperfusate solution. This has been successfully achieved using antineutrophil serums and leukocyte depletion (LD) filters. It is becoming common practice in many transplant facilities to leukocyte deplete blood in the reperfusate solution before exposure of blood to the new graft. This is often performed by incorporating an LD filter on the positive pressure side of the cardioplegia line. Literature suggests that 10 minutes is an adequate time of LD reperfusion before cross clamp removal to reduce the number of activated neutrophils in the ischemic organ (14). Heparin-coated circuits have also been found to decrease the inflammatory response associated with cardiopulmonary bypass (CPB) (15-17), thus decreasing the number of circulating activated neutrophils.

## CURRENT USES OF LEUKOCYTE DEPLETION

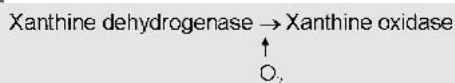
### Transfusion

The use of LD was originally used in blood banks to decrease risks of donor- associated transfusion reactions (18,19). Research has found that leukodepleted blood

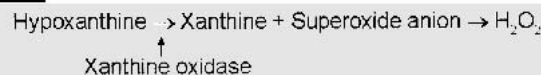
### Normal oxidation



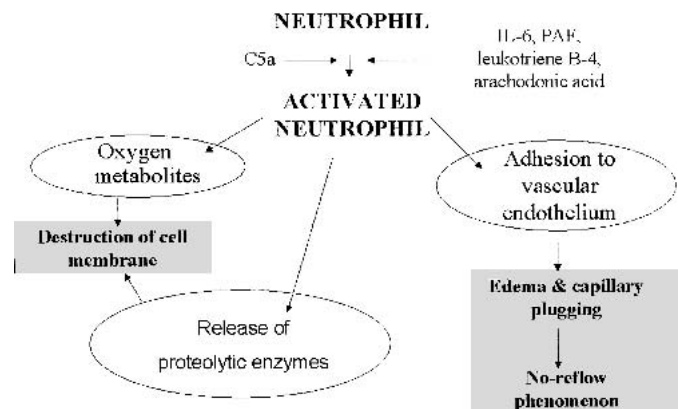
### Ischemia



### Reperfusion



**Figure 1.** Alterations in xanthine formation during ischemia and reperfusion and its role in oxygen free radical formation.



**Figure 2.** The activated neutrophil during cardiopulmonary bypass (1,5,7,8).

products help to decrease the incidence of post-operative infection and mortality compared to non-LD patients (20–22). Currently, many blood banks in the United States routinely leuko-reduce blood products before packaging. In fact, it is a federal law in Germany to use leukodepletion for all blood transfusions.

### Cardiac Surgery

Neutrophil activation commonly occurs in cardiac surgery because of contact activation when the patient's blood is exposed to the foreign surface of the CPB circuit (4,5). This predisposes the patient to neutrophil-mediated sequelae. LD has been used in the arterial line (23,24), venous line (25), and cardioplegia line (26) to help alleviate the devastating effects associated with leukocyte activation during bypass.

A study by Matheis et al. compared LD patients versus nonleukocyte-depleted patients during the reperfusion phase of CPB in 38 routine CPB surgery patients. This study aimed to optimize the onset and duration of leukocyte filtration during CPB. They found better outcomes postbypass with the use of LD during the early reperfusion phase of bypass as compared to systemic LD throughout surgery. Specifically, there was no need for catecholamines and a decrease in troponin-T levels postoperatively in patients undergoing LD selectively at reperfusion compared to other groups (27). They concluded that LD during early reperfusion might lessen myocardial damage endured by activated neutrophils during reperfusion. Additional studies of LD of blood cardioplegia agree with these findings (26,28).

A further study by Pala et al. observed the prevalence of oxidant-mediated damage after reperfusion with LD blood versus whole blood just before cross clamp removal. This study suggests that controlled reperfusion with LD blood reduced oxidant-mediated damage as compared to whole blood reperfusion ( $p < .05$ ) (29).

### Heart Transplantation

Pearl et al. studied the effects of LD reperfusion on the human heart during heart transplantation. Thirty-two patients undergoing heart transplantation were randomly divided into two groups. Group I was the control group, having normal whole blood reperfusion whereas, group II incorporated a Pall LD6 filter (Pall Corporation, East Hills, New York) into the cardioplegia line of the CPB circuit to be used during reperfusion. Hearts were reperfused with amino acid-enriched whole blood for 3 minutes and warm whole blood for an additional 7 minutes before cross clamp removal. Endomyocardial biopsies were reviewed for capillary swelling and interstitial edema at the end of ischemia and following reperfusion. It was found that LD reperfusion prevented ultrastructural damage; whereas, those patients not leukodepleted showed mild to

moderate interstitial edema and mild capillary endothelial swelling (30). This study suggests that LD may help ameliorate inflammation and capillary plugging of the new graft, which may have been caused by activated neutrophils during reperfusion.

Another study by Breda et al. analyzing reperfusion injury in the neonatal heart found similar results. This study observed 14 neonatal pigs undergoing heart transplantation. During the study, each pig underwent CPB, the heart was arrested using cold cardioplegia solution, and excised. The heart was then stored at 4°C for 12 hours in normal saline and re-implanted. Group I was reperfused with whole blood; whereas, Group II was reperfused with LD whole blood using an inline LD filter. The authors found a highly significant difference in myocardial function after reperfusion. Group I exhibited severe injury with myofibrillar necrosis, mitochondrial disruption, nuclear chromatin clumping, and moderate interstitial edema; whereas, Group II had normal myocardial ultrastructure ( $p < .0001$ ), indicating that LD helps mediate interstitial edema and myofibrillar necrosis (31). A similar study by Yamamoto using 10 canines showed similar results in cardiac function; cardiac function was significantly improved using LD reperfusion as compared to whole blood reperfusion ( $p < .05$ ) (32).

### Lung Transplantation

Graft dysfunction is estimated to occur in approximately 10–20% of all lung transplant recipients (33). It is believed that reperfusion injury plays a large role in graft dysfunction (34). In a study by Ross et al. (Charlottesville 1994), donor rabbits underwent lung harvest. Group I was reperfused immediately; whereas, Group II was isolated for 18 hours at 4°C and reperfused with whole blood, and Group III was also isolated for 18 hours at 4°C and reperfused with LD whole blood. They found that microvascular permeability was reduced in the LD group as compared to Group II. In addition, arterial oxygen saturation, pulmonary artery pressure, and pulmonary vascular resistance were significantly improved in the LD group as compared to Group II, suggesting LD helps protect against microvascular permeability and improve graft function (35). Further studies support these findings (36,37).

The transplant team at the University of California Los Angeles (UCLA) have successfully used LD during single lung transplant reperfusion with favorable results. A simple circuit was created using a 4:1 blood:reperfusate solution with the incorporation of an inline LD filter and heat exchanger on the positive side of the roller pump. Reperfusate solution consisted of glutamate/aspartate, tromethamine solution (THAM), citrate phosphate dextrose, and 50% dextrose. The lungs were reperfused at a flow not to exceed one L/min for a total of 10 minutes. Since the start of this study, 50 patients have been successfully transplanted with favorable results (38).

### Liver Transplantation

Ischemia reperfusion injury is a significant limitation to liver transplantation. Organ dysfunction is a direct result of the accumulation of activated neutrophils (39). These neutrophils seem to be recruited by the liver's release of tumor necrosis factor (TNF)- $\alpha$  in conjunction with vascular endothelial adhesion molecules (39,40). There has been one previous intra-operative study on the reduction of leukocytes during orthotopic liver transplantation (OLT). Parker et al. (Cleveland Clinic 2000) enrolled 21 patients with end-stage liver disease in a prospective, non-blind, historical control study of acute cellular graft rejection after OLT. They found that using leukocyte-reducing filters on all banked blood products and cell-salvaged blood significantly reduced the total amount of blood products used, as well as the incidence of acute cellular graft rejection (41).

During liver transplantation, LD may be accomplished through the veno-venous bypass circuit in institutions that have adopted this surgical method. Other means of leukoreducing the reperfusate include an autologous whole blood donation at the start of surgery, which can be reinfused through a LD filter during reperfusion. More research is needed to assess the benefits of LD during reperfusion in liver transplantation adequately.

### CONCLUSION

In conclusion, researchers have found evidence that suggests activated neutrophils play a large role in ischemic reperfusion injury. LD of the reperfusate may help to reduce the symptoms associated with ischemic reperfusion injury and, consequently, improve graft function. This is particularly evident in cardiac and pulmonary transplantation. However, benefits have also been shown in general cardiac surgery when ischemia is customary. More research may be necessary to assess the benefit of LD during other common transplant surgeries, such as liver and kidney transplantation.

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