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Pathophysiology of kidney reperfusion injury – An overview

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INTRODUCTION

A still increasing number of investigators are becoming attracted by the idea that it might be possible to reduce post ischemic renal failure by adequate treatment. This idea is based on the concept that ischemic damage is not definitively established at the end of an ischemic interval but is modulated by various processes during reperfusion. The possible additional damage is simply called reperfusion injury. Despite almost 10 years of elaboration of this concept in studies on renal ischemia, the importance of reperfusion injury has not yet been generally accepted. Indeed, progress is slow. A number of important questions are still to be answered. The prospect, however, of a method to reduce post ischemic renal failure makes it worth going on in view of the high incidence of acute renal failure in shock and sepsis, and the non- or late-functioning kidney transplants in everyday clinical practice.

In this review a search for evidence is presented that in the kidney post ischemic reperfusion injury is really an operative pathophysiological entity with substantial implications for clinical thinking and handling. The pathophysiology of renal ischemia is not discussed unless it is necessary for a better understanding of reperfusion mechanisms.

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KIDNEY

At a first glance one might expect no gross differences in ischemia-reperfusion injury among various organs. Intervention by ischemia and reoxygenation in such a fundamental cellular mechanism as the oxidative respiration might warrant a general theory on ischemia-reperfusion injury. How-ever, both at a metabolic level and at a three-dimensional structural level, i.e., anatomical and histological, properties are found, highly specific to the kidneys that certainly make the study and management of ischemia-reperfusion injury in the kidney different from other organs.

The kidney is a collection of individual but coordinated functioning units with a series connection of two capillary beds per unit. Each unit contains a large number of different, highly specialized epithelial cells. In this view the postischemic renal tissue should be considered as a mixed cell population with different degrees and types of injury. A number of cells may have gotten irreversibly damaged during the ischemic interval. Membrane disruption leads to a washout of the cellular content and there is no recovery of the energy metabolism. Cells may also die only some time after the onset of reperfusion, elicited by the challenge of reoxygenation and the renewed metabolic demand. Other cells remain viable. The energy metabolism recovers and possible membrane damage will get repaired. Finally, compensatory reactions occur in response to the reduction in renal mass. This mixture of events makes it obviously difficult to obtain a clear distinction between ischemic damage and reperfusion injury and may explain the large number of conflicting results among various studies.

Compensatory changes in functional as well as metabolic properties following reduction of renal mass as a result of ischemic injury are a well recognized and characteristic phenomenon in the kidney. As a result of the remarkable capacity to recover from extensive parenchymal necrosis after a long period of time, momentous changes in tissue integrity and renal function should be interpreted cautiously as they are not necessarily predictive for the final outcome of the recovery process [1].

A circumstantial advantage of the study of ischemia reperfusion injury in the kidney in comparison to e.g., the heart and the brain is the importance of the kidney to the viability of the organism as a whole. Acute renal failure threatens life only after some days. The time for the kidney to recover from ischemia-reperfusion injury is there for prolonged and can even be extended by artificial dialysis or the [temporal] presence of a well-functioning kidney. This option which is in common use in clinical practice, offers the opportunity to study the effect of even severe ischemic insults.

Ischemia and reperfusion may occur simultaneously in a single organ. Generally, total organ ischemia followed by total organ reperfusion with a clear starting point is rather exceptional. In the kidney, however, such a course of events is frequently encountered. In the kidney, however, such a course of events is frequently encountered. In the setting of kidney transplantation ischemia and reperfusion are distinct phenomena, clearly separated in time.

Many studies on postischemic renal damage have originally been initiated in the context of kidney preservation and transplantation and deal with global ischemia and total organ reperfusion.

REPERFUSION INJURY

The term reperfusion injury is often used synonymously with oxygen free radical injury. This is quite obvious if one considers that reoxygenation is the most prominent feature of reperfusion and the only way to stop hypoxia. Thus, the introduction of oxygen in the ischemic tissue is inevitable and similarly seems the generation of free radicals. The injurious effect of free radical formation has been recognized for almost 10 years and numerous studies have claimed the development of successful treatment strategies against this kind of injury. In studies on renal ischemia the term reperfusion injury has probably been used for the first time with respect to this postischemic oxygen free radical generation [2].

During ischemia there is supposed to be a conversion of the native xanthine dehydrogenase to a superoxide producing xanthine oxidase. The catabolism of high energy phosphates during ischemia provides an oxidizable substrate, hypoxanthine. Reperfusion delivers molecular oxygen leading to superoxide production. Symptoms of postischemic tissue damage can be reduced by inhibiting the working of xanthine oxidase with allopurinol treatment.

The real importance of free radical formation and the benefit of its treatment are still discussed. Different views are found in the literature on the source of the toxic radicals and their targets. In fact, there is only circumstantial evidence for the occurrence of free radicals since no methods are available to directly demonstrate their presence.

By now, the term reperfusion injury is used in a much broader sense including all pathophysiology mechanism that is confined in time and nature to reperfusion. The potential of this concept depends on that proportion of the total postischemic injury that is due to reperfusion mechanisms set against the proportion due to ischemia itself. Unfortunately, it is often difficult to make a clear distinction between reperfusion injuries as the ischemic injury that is caused by the very act of reperfusion. One might put an end to the inappropriate use of terminology by making a pragmatic distinction between avoidable and unavoidable damage. The first is called reperfusion injury and is defined by successful intervention studies.

A reliable parameter of ischemic injury would clearly facilitate the evaluation of experimental studies. The results of enzyme activity determinations in urine in this respect are generally disappointing. The transport of released enzyme is probably hampered by postischemic tubular flow disturbances and a rapidly changing urinary flow. These problems may in part be circumvented by correlating urinary enzyme release to changes in glomerular filtration rate [3]. Other studies suggest that only minor quantities of the total amount of tissue enzyme activity that is lost are transported to the urinary system, whereas the enzyme activity is almost

completely recovered in the plasma. Nevertheless, changes in plasma enzyme activity during postischemic reperfusion have been considered small and hence of limited diagnostic importance. This can be explained by the appearance of renal enzymes in the circulation which is characterized by a rapid and very temporal release and which will only be found by early and frequent plasma sampling following the onset of reperfusion. By calculating the cumulative amount of enzyme activity in the circulation in this way, a reliable estimation of the total loss of enzyme activity can be obtained. This technique is rather elaborate and may be difficult to establish in the clinical setting [4, 5].

Monitoring changes in high energy phosphate metabolism for evaluation of renal ischemic injury has been advocated for almost 30 years. Sophisticated techniques like HPLC and 31p NMR has been used to study the behavior of these metabolites. Surprisingly little progress has been made in those 30 years. The catabolic pathways of the high energy phosphates during ischemia were extensively described by German investigators in 1963 and still appear correct. Unfortunately, the diagnostic and viability predicting value of the tissue content of high energy phosphates is often limited as will be discussed later.

MECHANISMS OF REPERFUSION INJURY

VASCULAR OBSTRUCTION/CONGESTION

An inhomogeneous distribution of blood or a perfusate throughout the renal tissue during reperfusion leads to sustained ischemia in the unperfused areas. The trapping of blood elements or local cellular fragment and edema formation are the most likely causes of an inadequate perfusion. Especially in early organ preservation research this concept has received much attention.

Post ischemia fibrin deposits arise in bowman's space, tubules, and peritubular capillaries and may thus be involved in edema formation. This phenomenon is not observed if 5 min before the ischemic insult heparin is administered [6]. Heparin treatment was advocated many years in renal preservation. Its effectiveness in clinical practice has been questioned. Nowadays it is used only in special cases.

Studies using ^{51}Cr labeled erythrocytes demonstrate that during 20 min of reperfusion a vascular congestion with erythrocytes occurs in both warm and cold ischemic kidney. The amount of erythrocytes being trapped is to some extent related to the length of the ischemic interval. The trapping is most pronounced in the inner stripe of the outer medulla [7]. This phenomenon probably refers to what has been called "the blue line", a macroscopically striking change in color of the outer medulla in postischamic kidneys. Vascular congestions have also been related to cell swelling. The swelling of the cells depletes the interstitial and vascular space, and occludes the tubular lumen.

The question is whether erythrocyte trapping or cell swelling in the outer medulla is merely an epiphenomenon of cell damage or by itself a cause of damage. Vascular congestion can be counteracted by hemodilution. Following 60 min of ischemia hemodilution started before reperfusion completely prevents vascular congestion when evaluated at 24 hr in rat experiments. Tubular cell necrosis, however, remains unaffected. In these studies the outer stripe of the outer medulla is suggested to be the major site of congestion. Mannitol infusions given before an ischemia unaffered. In these studies the outer stripe of the outer medulla is suggested to be the major site of congestion [8]. Mannitol infusions given before an ischemic insult prevent congestion by reduced cell swelling. In addition mannitol also improves postischemic renal recovery [9]. The osmotic effect of mannitol is obviously responsible for the reduction of cell swelling, but what about the radical scavenging effect of mannitol? Since hemodilution prevents congestion but fails to improve functional recovery the improvement of renal recovery observed following treatment with mannitol might be attributed to the scavenging effect rather than to the reduction of cell swelling. If this hypothesis holds true a major contribution of vascular congestion/obstruction per se to postischamic injury has not yet been established.

The accumulation of toxic metabolic products that arise during the ischemic insult may cause local injury and may be involved in edema formation and congestion but also in the stimulation of an inflammatory response. Early cells which have been subjected to ischemia. The disturbed metabolic homeostasis is challenged by a sudden high energy demand. Surprisingly, the energy metabolism of the kidney cells is able to adapt rapidly to renewed normoxic condition even after long term ischemia. After an ischemic interval of 30 min the energy charge in canine kidneys is back at preischemic levels within 5 min of reperfusion and replenishment of the high energy phosphate pool starts immediately. Within 1 hr of reperfusion tissue high energy phosphate levels have almost returned to preischamic levels. Following prolongation of the ischemic interval to 90 min the rapid normalization of the energy charge is still present. The increase of the energy phosphate pool, however, is much more retarded [10]. In the rat the renal high energy phosphate content apparently does not increases within the first 15 min of reperfusion following 25 min of ischemia [11]. Others found a doubling at 60 min of reperfusion following 50 min of ischemia [12]. At 24 hr the postischamic ATP content remains depressed only following a severe ischemic insult [60 to 90 min] [13].

The tissue content of high energy phosphates corresponds to postischamic functional parameters, e.g., glomerular filtration rate as was demonstrated again recently [14]. However, evidence that the energy phosphate content per se dose not reflect the potential functional capacity of the postischemic kidney has been obtained in studies on the effect of exogenous supply of metabolites. It is now well established that the energy phosphate content can be increased with the use of pharmacological agents. In some studies treatment with such agents has been successful in improving functional renal parameters. In other studies, improvement of function has been achieved without notable changes in the tissue energy phosphate content and increases in tissue energy phosphate content have been achieved without any beneficial effect on renal function [15-19].

There may be an explanation for these conflicting results. If one considers the xanthine oxidase Mediated hypoxanthine xanthine conversion as a major source of oxygen free radicals then the exogenous administration of high energy phosphates may enhance this reaction by indirect supply of hypoxanthine. This hypothesis is supported by studies in which it is shown that following the addition of exogenous hypoxanthine and xanthine to the perfusate of isolated perfused rat kidneys total organ flow and glomerular filtration rate are dramatically reduced. Tubular reabsorption properties, however, remain undisturbed. Since pretreatment with SOD and catalase abolishes these effects the exogenous hypoxanthine and xanthine are likely to induce free radicals [20]. It must be noted that the flow alterations can be explained by a direct effect on vascular resistance. Hypoxanthine may be converted to familiar metabolites which are strong vasoactive compounds that are able to induce vasoconstriction [21, 22].

XANTHINE OXIDASE

After a certain time interval following the onset of the ischemic insult the high energy phosphate pool is no longer kept constant and starts to decrease. By the breakdown of AMP a spectrum of degradation products is generated which shows some specificity to tissues and species. If the ischemic insult persists the breakdown of AMP will theoretically proceed to xanthine and uric acid. The activities of the enzymes that are involved in different catabolic steps apparently show a different sensitivity to the effects of ischemia. As a result some degradation products definitively accumulate whereas others are only transiently increased. In the kidney a limited accumulation of inosine and adenosine is found whereas in heart tissue these products show a profound increase during ischemia. Hypoxanthine is by far the most important degradation product in ischemic kidneys. The characteristic accumulation of hypoxanthine has been recognized as early as 1945 [23]. In the dog, the rabbit, and the man the hypoxanthine level steadily increases according to the length of the ischemic interval [24-27]. In the rat a somewhat different pattern is observed. The hypoxanthine accumulation is less pronounced whereas a concomitant and equal accumulation of xanthine is found [27]. The conversion of hypoxanthine to xanthine is mediated by the enzyme that is supposed to be involved in the generation of oxygen free radicals during ischemia as was pointed out before. The tissue content of xanthine oxidase and of the endogenous oxygen free radical scavenger varies among different species. Whether this variation determines ischemia tolerance by specific sensitivity to oxygen free radicals or rather weakens the putative important role of xanthine oxidase is the subject of speculation.

The beneficial effect of allopurinol on renal recovery from an ischemic insult support the role of the high energy phosphate catabolism in the generation of free radicals since the effect of allopurinol is considered to be based on the inhibition of the hypoxanthine-xanthine conversion. Postischemic lipid peroxidation is effectively reduced by alloporinol treatment. The effect is most pronounced when alloporinol is administered before the onset of reperfusion rather than when administered before the onset of the ischemic interval. The inhibition of xanthine oxidase during reperfusion seems more important than the prevention of high energy phosphate breakdown during ischemia. Evidence in support of this argumentation is weak. The

pharmacokinetics of allopurinol during ischemia is unknown and the exact relationship between high energy phosphate breakdown and xanthine oxidase activity is also unknown.

An unusual way to study the important of free radical generation by xanthine oxidase is to experiment with tungsten. If animals are fed with tungsten for weeks a renal tissue depletion of xanthine oxidase is obtained. Subsequently, the renal tissue is more tolerant to ischemic insults. A more direct approach is found in studies on the effects of the free radical scavengers SOD and catalase which are naturally present in renal tissue. Exogenously administered human bovine SOD have been shown to be effective in enhancing postischamic renal recovery. Dimethylthiourea, a hydroxyl scavenger, and SOD induce a reduced increase in serum creatinine levels and less signs of tubular necrosis after 24 hr are seen in the rat. Long term results of SOD and catalase treatment of ischemic kidney have been reported. Five days after ischemic insult serum creatinine levels are still reduced to almost 50% in comparison to untreated animals [pigs].

Even since the tissue content of xanthine oxidase appeared to be tissue and species dependent its important role in free radicals generation has been questioned. Recent studies in the rat did not confirm a beneficial effect of xanthine oxidase activity inhibition of postischemic renal injury. In recent enzyme studies in the rat no conversion of xanthine dehydrogenase to xanthine oxidase was found despite the presence of lipid peroxidation. Apparently, our theoretical concept still lacks some important facts.

Metabolic subtracts have been supplied in order to alter the preceeding of putative destructive metabolic pathways. The enhancement of anaerobic carbohydrate utilization by administration of fructose-1, 6-diphosphate before the ischemic insult or during reperfusion imporoves post ischemic renal recovery. There is some evidence that the effect of fructose-1,6-diphosphate is due to inhibition of oxygen free radical generation.

LIPID PEROXIDATION

The study of lipid peroxidation in ischemia reperfusion injury reveals further information on the role of free radical generation during reperfusion and provides insight to the mechanisms leading to loss of member integrity.

Lipid peroxidation is characteristically related to the oxygen tension in is chemically damaged renal tissue and is therefore strictly associated with reperfusion. There is hardly any evidence for the occurrence of lipid peroxidation during the ischemic interval. At the end of a 120 min ischemic interval some diene conjugates have been found in rabbit kidneys, Other parameter of lipid peroxidation could not confirm these finding. During cold ischemic some lipid peroxidation occurs but in no proportion to the burst of peroxidation that is found immediately following the onset of reperfusion after cold storage. Evidence of lipid peroxidation as measured by malondialdehyde levels in venous effluent is found immediately following the onset of reperfusion after 45 min ischemia in the rat. After 5 min the malondialdehyde recovery ceases and dose not return within another 85 min. evidence of lipid peroxidation as measured

by ethane levels in expired gas shows a somewhat different pattern. Not until the beginning of reperfusion is a significant increase of ethane observed which persists for 50 min. Base line levels are rapidly reached again. The ethane production is prohibited by both allopurinol and SOD. A reduction of lipid peroxidation is also obtained by the inhibition of cyclooxygenase using indomethacin. The beneficial effect is especially noted in the renal medulla. A sustained long term effects on postischamia renal function by reducing lipid peroxidation has not yet been demonstrated.

CALCIUM HOMEOSTASIS

Calcium entry blockers reduced lipid peroxidation. NMR techniques reveal a rapid rise of intracellular Ca^{2+} content to control levels whereas stimulation of Ca^{2+}transport exacerbates postischamic injury. The calcium entry blockers, Verapamil and Necardipine, both improve functional, histological, and metabolic properties of canine kidney following 60 min of ischemia leading to an increased animal survival in unilateral nephrectomized dogs. Verapamil in the preservation fluid of cold stored kidneys improved tubular function and glomerular filtration rate and increases tissue ATP levels when evaluated at 1 hr of reperfusion in isolated perfused kidney studies. In clinical renal transplant studies Ca^{2+} entry blockers generally improve functional parameters early postoperative. Such an effect is even obtained if the blockers are administered after the ischemic insult. No evidence is yet available as to whether or not long term graft survival is improved.

When renal mitochondria are loaded with Ca^{2+} in the presence of oxygen free radicals, mitochondrial injury is potentiated. The activation of phospholipase A2 by Ca^{2+} may be involved in this process. The addition of xanthine oxidase to isolated mitochondrial in *in vitro* induces a Ca^{2+} release. This efflux is only blocked by the administration of a catalase. Superoxide dismutase has not effect, suggesting that the damaging radical products are produced by the H_2O_2 metabolism. *In vitro* studies provide evidence that exogenous ATP prevents radically induced mitochondrial Ca^{2+} efflux in a dose dependent way. *In vitro* studies have shown that after 50 min of renal ischemia in the rat a passive mitochondrial Ca^{2+} accumulation occurs, probably as a result of a progressive increase of the cytosolic Ca^{2+} concentration. Early reperfusion Phase is characterized by a normalization of the mitochondrial Ca^{2+} uptake, release, and steady state buffering. Recent studies also suggest, however, that the working mechanism of Ca entry blockers might be confined to their vasodilator properties rather than to a protection of cellular membrane integrity.

IRON

Two other system that are involved in oxygen free radical generation and lipid peroxidation have been studies in the kidney, free iron with its chelators and the glutathione peroxidase system.

In ischemic renal cortical tissue a decompartmentalization of intracellular iron occurs and a proportion increase in free iron. An increase in free iron is supposed to enhance the oxygen free radical mediated lipid peroxidation since iron when exogenously administered enhances lipid peroxidation and increases renal failure. On the other hand, chelation of free iron induces a reduction in lipid peroxidation and decreases postischemic injury. The iron chelator desferrioxamine reduce lipid peroxidation in rabbit kidneys subjected to 60 and 120 min of ischemia when administered 15 min before reperfusion. Free iron is not detected in the venous effluent following reperfusion but is found in the urinary system. This is consistent with findings that iron chelation offers only protection if it reaches the tubules.

GLUTATHIONE

The glutathione peroxidase system metabolizes hydroxyl peroxides. According to the glutathione redox ratio [{GssG/(GSH+ GssG)}.100] the oxidant stress during reperfusion is transient within 15 min after the end of a 40 min ischemic interval. Dimethylthiourea infusions during reperfusion attenuate glutathione consumption. Both glomerular filtration rate and tubular Na reabsorption are improved following 20 to 30 min of ischemia by dimethylthiourea treatment. Following 45 and 60 min of ischemia, however, no functional improvement is observed although the glutathione consumption is still attenuated. Glutathione has been administered to ischemic renal tissue to support the endogenous radical scavenging process. Numerous negative side effects, however, hamper fruitful use of the metabolite in this way.

INTEGRITY OF THE MEMBRANES

The target of the toxic oxygen metabolites that may be produced during reperfusion is hard to define. In general, the viability of cell is directly related to the integrity of their membranes. In addition to the the effect on renal mitochondria as demonstrated in *in vitro* studies one might expect that radicals induce directly or indirectly changes in membrane function and integrity. A derangement of the Na-K pump is an early sign of the cell in distress.

Many studies claim that proximal tubular damage is an essential part of ischemia reperfusion injury. An ischemic insult of 15 min and 2 hr of reperfusion induce an abnormal redistribution of Na-K ATPase from the basolateral membrane to the apical membrane. Simultaneously, the membrane lipid composition changes consistent with a loss of surface membrane polarity. Notably, the ultra structure of the proximal tubular cells remains intact after 15 min of ischemia. Thiazide diuretic receptors kidney membranes are down regulated following 10 min of ischemia and 10 min of reperfusion. The down regulation of the membrane receptor, however, might occur during the ischemic interval. In microsomal fractions prepared from the outer medulla of canine kidney Na-K ATPase activity is significantly decreased after 1 hr reperfusion and 1 hr of ischemia.

In vivo studies in the dog show, besides a reduction in Na-K ATPase activity, an increase in microsomal lipid peroxidation and an increase in malodialdehyde levels in the venous

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effluent. These changes are largely prevented by infusions of glycine which is known to counteract free radical generation.

MECHANISMS OF LYMPHOCYTE ACTION DURING KIDNEY ISCHEMIA–REPERFUSION INJURY

Kidney ischemia–reperfusion injury (IRI) is characterized by endothelial dysfunction, sub lethal injury to renal tubule epithelial cells, and an increased production of chemokines, cytokines, and oxygen free radicals that amplifies cell damage. Exposure of antigens in the kidney after IRI activates antigen-presenting cells (APCs) that migrate to the draining lymph nodes and present the antigen–MHC II complex to T lymphocytes using specific T-cell receptor (TCR) recognition. Activation of lymphocytes requires costimulatory molecules. Activated T lymphocytes infiltrate into kidney from blood, recruited by chemokines into the damaged kidney (Fig.1).

Lymphocytes, neutrophils, macrophages, and platelets physically contribute to microvascular sludging and 'no-flow.' Microvascular adhesion, signaling, and transmigration into parenchyma are mediated by adhesion molecules, which further amplify inflammation and cytotoxic injury. T lymphocytes, both infiltrating and resident, can also be activated directly in situ by reactive oxygen species and chemokines or as a result of direct contact with tubular cells. CD40, present on tubular cells, interacting with CD40 ligand (CD40L) on T lymphocytes, leads to the production of chemokines by the epithelial cells with activation and further recruitment of leukocytes.

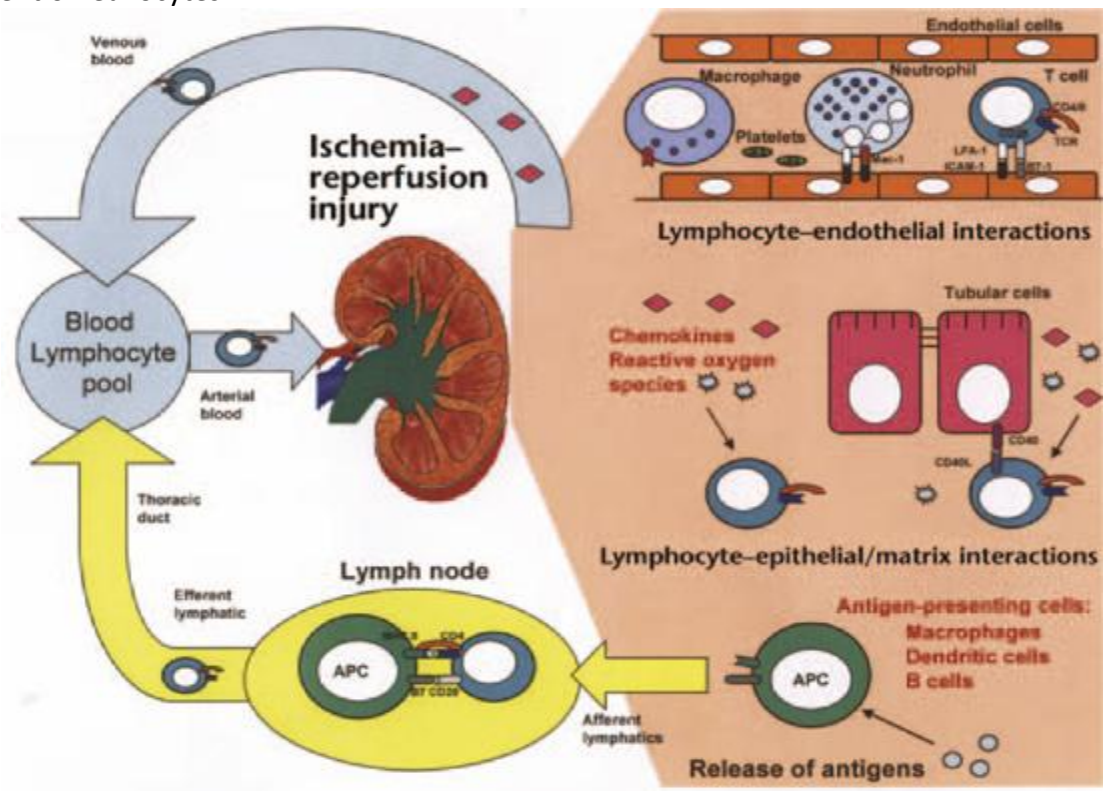

Figure 1. Mechanisms of lymphocyte action during kidney ischemia–reperfusion injury.

Lymphocyte function–associated antigen-1 (LFA-1); intercellular adhesion molecule-1 (ICAM-1); MHC, major histocompatibility complex.

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