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Ischemic acute tubular necrosis models and drug discovery: a focus on cellular inflammation

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Short Running Title

Ischemic kidney model and inflammation

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Abstract

Acute renal failure (ARF) is a common cause of mortality and morbidity in hospitalized patients. Ischemia is an important cause of ARF and ARF due to ischemic injury is traditionally referred to as ischemic acute tubular necrosis (ATN). There is growing evidence that ischemic ATN is associated with intra-renal inflammation using ischemic ATN models. Consequently, intra-renal inflammation is an attractive target for the development of novel drug therapies for ARF. In this review, we will outline ischemic ATN models, the pathophysiogical roles of inflammatory cells such as T and B cells in ischemic ATN models, and effective T and B cell therapeutic reagents.

Introduction

Acute renal failure (ARF) is a common clinical syndrome and the manifestations result from a decline in glomerular filtration rate (GFR) and retention of nitrogenous waste products. There is currently no specific therapy for ARF except for supportive care. ARF is generally classified into three categories: (a) prerenal ARF characterized by decreased renal perfusion; (b) intrinsic renal ARF mostly accompanied with acute tubular necrosis (ATN); (c) postrenal ARF caused by an obstruction to urine flow. It should be noted that these three types of ARF are not mutually exclusive, and any two or all three of them may be present at the same time.

Ischemia is an important cause of intrinsic renal ARF and ARF due to ischemic injury is traditionally referred to as ischemic ATN. There is growing evidence that ischemic ATN is associated with intra-renal inflammation using in vitro and in vivo models for ischemic ATN. Initially, many researchers focused exclusively on the neutrophil as the cellular mediator of intrarenal inflammation because neutrophils are key components of innate immunity and neutrophil migration into renal tissue after ischemic injury was observed with the experimental models. However, the most studies have had mixed results studying effect of neutrophil depletion or blockade on ischemic ATN [1-3] and therefore the neutrophil is now thought to play a modest role in the course of ischemic ATN. Macrophage migration into renal tissue and the upregulation of chemoattractants (e.g., monocyte chemoattractant protein-1) for macrophages have also been seen in the kidney of experimental ATN models [4-6], but there is limited evidence concerning the role of macrophages in ischemic ATN [7]. Although the importance of T cells in ischemic ATN was not recognized for many years because of the sparse distribution of T cells in the kidney during ischemic ATN, accumulating evidence within the past few years strongly supports a role for T cells as modulators of

ischemic ATN [8-10]. Furthermore, recent observations have indicated that T and B cell interaction is involved in ischemic ATN [11, 12]. Due to the feasibility of using well-characterized T cell therapeutic drugs in man, the precise definition of the roles of T and B cells opens up new opportunities for drug discovery against ARF. In this review, the pathological roles of inflammatory cells such as T and B cells in ischemic ATN are addressed with an overview of the experimental ischemic ATN models. Moreover, pharmacological interventions which have recently been identified and are thought to be mediated by acting on T and B cells are described.

In vitro models

In vitro models for investigating ischemic ATN include isolated perfused kidneys, freshly isolated tubules, and cultured tubular cells. These models have been developed to gain mechanistic insights into ischemic ATN in *in vivo* animal models and humans (Table 1).

Isolated perfused kidney

In this model, the kidney is isolated from the animal (studies have mainly used isolated rat kidneys) and is perfused with an oxygenated solution through the renal artery [13,14]. The GFR, tubular function as well as renal morphology can be assessed. The model is divided into two types according to perfusate used, such as erythrocyte-free and erythrocyte-containing perfusate [15, 16]. The most remarkable difference between the two models is the site of tubular injury. In the presence of erythrocytes in perfusate, ischemia produced by lowering the oxygen concentration in perfusate causes injury in proximal tubules (S3 segments), but not in the medullary thick ascending limb (MTAL) of the distal nephron. In the absence of erythrocytes in perfusate, MTAL injury progressively occurs without ischemic insult. This spontaneous tubular injury in MTAL is inhibited by the addition of amino acids to the re-circulating perfusate [17]. The model in the presence of erythrocytes is closer to human ATN and animal *in vivo* ARF models, while the model without erythrocytes is simpler and reduces complexity of the system.

Freshly isolated tubules

There are two types of freshly isolated tubule models; isolated tubules with microperfusion and suspensions of isolated tubules.

The method for isolated tubules with microperfusion was originally developed by Orloff and colleagues [18]. The tubules are dissected from a slice of kidney with fine forceps by hand and the isolated tubules are then microperfused with precision glass pipettes. Although this experiment requires much skill, it is a powerful tool for investigating the transport characteristics of multiple nephron segments in an ischemic condition. Hanley [19] showed with this technique that the most serious tubular transport alterations in response to ischemia were found in proximal tubules compared to cortical TAL and cortical collecting tubules.

A renal tubule suspension technique has been developed to circumvent disadvantages of classical tissue slice preparations such as the insufficient diffusion of oxygen to the innermost regions and, in addition, the closure of the tubular lumen [20,21]. A renal tubule suspension is prepared by collagenase digestion and the collagenase-separated renal tubules have a well-conserved morphology and open tubular lumina. Furthermore, the delivery of sufficient oxygen to this tubule suspension is easily performed. Although this model has been widely used to gain mechanistic insights underlying ischemic tubular injury, it is important to note that isolation damage is inevitable in a portion of cells.

Cultured tubular cells

Two types of cell cultures have been extensively used for investigating cellular mechanisms underlying ischemic ARF: primary cultures from proximal tubules [22] and continuous LLC-PK1 cell lines (http://www.atcc.org/common/catalog/numSearch/numResults.cfm?atccNum=CL-101) originated from swine proximal tubules. Although isolation damage can be avoided with cultured tubular cells, it must be kept in mind that phenotypic changes of cultured

tubular cells occur compared to freshly isolated tubules.

In vivo models

Because of the difficulties in examining pathophysiology of human ischemic ATN and of the discovery of therapeutic interventions for ARF, many animal models have been devised. In this section, four models are described; the renal ischemia-reflow model, whole body ischemia-reflow model, toxic model, and glycerol-induced ARF model.

Renal ischemia-reflow model

Renal ischemia-reflow models are divided into two models, one warm and one cold ischemia-reflow model.

Warm renal ischemia is the most widely used experimental model to investigate the pathophysiology of human ischemic ATN [23, 24, see related articles]. The renal vascular pedicle of mice or renal artery alone of dogs or rats is clamped for a variable length of time and subsequently the kidney is reperfused. The severity of injury depends on the time of obstruction. Controlling the body temperature of the animal is very important, because lower temperatures reduce the severity of injury. In this model the initial insult is caused by hypoxia to the tissue which is then followed by altered microcirculation. Inflammation and reactive oxygen species formation are also involved in the progression of tubular injury. In this model, S3 segments of proximal tubules are mainly damaged, while distal nephron involvement is minimal. This model is very simple and reproducible. In addition, there are several similarities between this model and human ischemic ATN which include severe reduction in GFR, injury to the proximal brush border, and the presence of cast formation. However, several concerns have been expressed over the use of this model. Complete cessation of renal perfusion is

uncommon as a cause of human ischemic ATN. Furthermore, S3 segments of proximal tubules are less prominent and MTAL damage may be much more severe in human ischemic ATN than in warm ischemia-reflow model.

In cold renal ischemia-reflow, the kidney is removed, flushed, kept at a low temperature, and then reimplanted. Harvig et al. [25] have reported using this rat model that tubular necrosis is sparse in the proximal tubules and is extensive in the inner stripe and inner zone of the renal medulla. This contrasts with the injury pattern of warm renal ischemia-reflow model.

Whole body ischemia-reflow model

In most cases of human ischemic ATN, reperfusion of the kidney occurs after whole body ischemia. Approximately 30% of patients who are resuscitated from in hospital cardiac arrest developed ARF. Our group has devised a new murine model of ARF after whole body ischemia [26]. To cause cardiac arrest, cold KCl solution (0.5 M, 2.8 μl/g body) is injected into mice through the jugular vein and at 570 sec after cardiac arrest, artificial respiration begins. Thereafter, epinephrine injection and cardiac massage are performed. If spontaneous circulation does not restore by 12 min after cardiac arrest, resuscitation efforts are stopped. This model clearly shows ARF events in terms of serum creatinine, tubular injury, and inflammation.

Toxic model

Some chemicals are known to directly cause renal tubular injury. Nephrotoxic agents including gentamycin-an aminoglycoside antibiotic, and cisplatin-a chemotherapeutic agent, have been widely used to produce animal models simulating tubular cell death.

The parenteral treatment of animals (mainly rats) with gentamycin (100 to 200

mg/kg BW) is repeated for 3 to 6 consecutive days [27]. Gentamycin-induced ARF is reversible and a recovery phase is comparable with that of the human gentamycin nephrotoxicity.

Cisplatin is given once to rats or mice (6-40 mg/kg) intraperitoneally to cause a direct tubular nephrotoxicity [28]. The model is very simple and cisplatin predominantly injures S3 segments of proximal tubules in animals. This injury pattern is comparable with that seen in humans in response to cisplatin.

Glycerol-induced ARF model

Intramuscular injection (hind limb muscle) of hypertonic glycerol (50%, 8-10 ml/kg) induces rhabdomyolysis and a form of ARF in rats [29]. Glycerol-induced ARF is thought to be caused by complex factors such as dehydration, intrarenal vasoconstriction, heme-mediated reactive oxygen species production, and cast formation. Because multiple segments injury has been observed, it has been proposed that the glycerol-induced ARF model is a relatively satisfactory model for human ATN.

In silico models

Many cellular events are involved in ischemic ATN, such as necrosis, apoptosis, proliferation, migration, and differentiation of cells. These cellular responses are driven by many molecules. Therefore, the molecular dissections of ischemic ATN are required to fully understand its mechanisms. To this end, comparative genomics, functional genomics, proteomics, and bioinformatics have been employed. However, studies with these techniques are in incipiency (Table 1). In this section, we show some data with genome-wide analysis of ischemic ATN in animal models and humans.

One of the initial studies was performed by Ichimura et al [30]. A rat warm renal

ischemia-reflow model was used with a technique called representational difference analysis (RDA). RDA is a PCR-based method to evaluate differences in gene expression. They observed the up-regulation of kidney injury molecule-1 (KIM-1) after renal ischemic reflow. KIM-1 was also identified in human kidney biopsy and urine [31-33].

Some groups have utilized cDNA microarray technology to provide parallel and quantitative expression profiles of thousands of genes (eg., http://www.ncbi.nlm.nih.gov/geo/ with accession no. GSE1714). Devarajan and co-workers [34] have screened for changes in expression of 9,000 sequence-verified mouse genes at several points following warm renal ischemia-reflow and have found several novel genes that were up-regulated during early warm ischemic injury. Among them, neutrophil gelatinase-associated lipocalin (NGAL) has been further characterized. NGAL protein expression levels were dramatically increased in the early post-ischemic mouse kidney and human acute renal injury after cardiac surgery [35]. Furthermore, intravenous NGAL administered before or after an ischemic insult ameliorated the warm ischemia-reflow-induced renal injury in mice [36].

Kieran et al. [37] have found that 445 of a total of 12,488 genes were altered more than two-fold 24 hours post ischemia with a microarray technology and analyzed unknown genes with a bioinformatic annotation pipeline. The altered genes were classified under three categories, including known genes previously implicated in the ischemic ATN (e.g., KIM-1, intracellular adhesion molecular-1, p21), known genes not previously related to ischemic ATN (e.g., claudin-1, -3, and -7), and uncharacterized genes (e.g., enigma proteins).

Genome-wide gene-expression analyses using cDNA microarrays in human kidneys have also been performed [38]. Oberbauer and co-workers (http://www.akh-

wien.ac.at/nephrogene/) checked both 26,338 genes and 14,783 Expressed Sequence Tags (ESTs) in recipients of cadaveric donor kidneys with or without ARF (microarray data can be accessed at http://genome-www5.stanford.edu/cgi-bin/publication/viewPublication.pl?pub_no=397). ARF upregulated 48 genes and those functional roles could be classified into cell cycle regulation, cell metabolism, signal transduction, and no defined function. Interestingly, they showed that protein kinase CK2 (formerly known as casein kinase II) was up-regulated by ARF and this kinase was recently reported by another group [39] to play an important role in the progression of glomerulonephritis using cDNA microarrays (microarray data can be accessed at www.ncbi.nlm.nih.gov/geo with accession no. GSE1262), suggesting the importance of protein kinase CK2 in a broad range of kidney diseases.

Inflammatory cells in ischemic ATN models

In the past few years there has been remarkable progress in recognition for the roles of inflammatory cells such as T and B cells in ischemic ATN (Fig. 1), using the above-mentioned ischemic ATN models combined with transgenic and knock-out animals. In this section, the pathogenesis of ischemic ATN is discussed with an emphasis on the evidences supporting a role for lymphocytes in experimental ARF.

Although T cells were not thought to be associated with ischemic ATN according to the classic models of innate immunity, CD3 positive T cells could be visualized in human cadaveric kidneys with ischemic ATN in the absence of rejection or calcineurin inhibitor toxicity [10]. T cells have also been identified in warm renal ischemia-reflow rat and mouse models. Furthermore, amelioration of tubular damage induced by warm renal ischemia-reflow has been observed in double CD4/CD8 knock-out, single CD4 knock-out and T-cell-deficient (nu/nu) mice. These data substantiate the

important role of T cells in ischemic ATN [22, 40].

$CD4^+$ T cells functionally differentiate into two phenotypes, T helper (Th)1 and Th2 cells. Th1 cell differentiation occurs efficiently when interferon (IFN)-γ and interleukin (IL)-12 act together, requiring both signal transducer and activator of transcription 1(Stat1) and Stat4. The Stat6 and IL-4 promote Th2 differentiation. We investigated the effects of reconstituting nu/nu mice with $CD4^+$ T cells from IFN-γ-deficient mice (B6.129S7-*Ifng*^{tm1Ts}) and observed that IFN-γ-deficient $CD4^+$ T cells did not worsen the injury in response to warm ischemia-reflow [40]. This strongly suggested that the Th1 phenotype was associated with ischemia-induced renal injury. This hypothesis was extended by the use of experiments with either Stat4 or Stat6-knock-out mice. It is interesting to note that Stat6-deficient mice had markedly worsened renal function by ischemia compared with wild type, suggesting that Th2-related signals had a protective role in ischemic ATN [41].

Given that T and B cells have a mutual effect in transplant rejection and asthma, we hypothesized that T and B cell interaction played a role in ischemic ATN pathobiology. B cell-deficient mice (*Igh-6*^{tm1Cgn}) subjected to warm ischemia-reflow had a reduced renal injury as compared with that in wild-type mice [11]. However, warm ischemia-reflow induced renal injury was not ameliorated in recombinase activating gene-1 (RAG-1) deficient mice, which lack both T and B cells [12]. These findings may have been due to the enhanced NK cell activity in these mice. These results demonstrate that the B cell is a type of mediator in ischemia-reflow injury and that an interaction of T and B cells likely occurs in ischemic ATN. In order to determine a precise role of each cell type, further studies are required.

Drug discovery for ischemic ATN

As noted in the previous section, T and B cells play an important role in the pathobiology of ischemic ATN. In this section, in order to steer drug discovery regarding intra-renal inflammation toward human ischemic ATN, T and B cell reagents recently identified that can alter the course of experimental ischemic ATN are described (Table 2).

As inflammatory cell activation is preceded by the up-regulation of soluble mediators of inflammation such as cytokines and chemokines, these mediators are potential targets for novel drug therapies against ARF. Deng et al. [42] have shown that IL-10, a pleotropic cytokine with many immunosuppressive effects, protects against warm and cold ischemia-reflow-induced and cisplatin-induced renal injury. Because IL-10 produced by Th2 cells, is known to inhibit cytokine synthesis by Th1 cells in the presence of monocyte/macrophage antigen presenting cells and as mentioned earlier Th1 phenotype is deleterious in a warm renal-ischemia-reflow model, IL-10 is likely to protect ischemic ATN via the inhibition of Th1-related signaling pathway. A recent observation with anti IL-12 antibody, which is expected to inhibit the process of Th1 cell development in some way, supports this notion [43].

IL-6 was originally identified as a cytokine inducing B cell maturation. Given that renal damage after ischemic insult is, in part, mediated by B cells, inhibition of IL-6 would be expected to protect ischemic ATN. Recently, a study by Patel et al. [44] revealed that IL-6 knockout ($IL\text{-}6^{-/-}$) mice and mice treated with a monoclonal antibody against IL-6 were protected from warm ischemia-reflow-induced renal injury.

Adhesive interactions between the vascular endothelial cells and leukocytes initiate the infiltrate of leukocytes to sites of inflammation. The selectin family including P-, E-, and L-selectins is largely involved in this adhesion (rolling). Bimosiamose (TBC-1269), a novel synthetic inhibitor for all selectins, exerted

protective effect on tissue injury in warm renal ischemia-reflow model [45]. In addition, Langer et al. [46] showed that bimosiamose inhibited renal tissue injury and allograft rejection in cold renal ischemia-reflow model. As Th1 cells are known to express P-selectin, it is likely that the inhibitory effect of bimosiamose on renal injury is mediated by the reduction of binding capability of P-selectin on Th1 cells to P-selectin ligand.

CD28 on T cells can bind to both B7-1 (CD80) and B7-2 (CD86) on activated antigen-presenting cells and this interaction causes the T cell proliferation. Both CTLA-4 and CTLA-4 Ig, a fusion protein having the extracellular region of CTLA-4, are known to act as a potent competitive inhibitor of an interaction of CD28 with B7, resulting in inhibition of T cell proliferation. CTLA-4 Ig or a combination of anti-B7-1 and B7-2 antibody has been shown to have some protective effects on both cold and warm ischemia-reflow-induced renal injury in rats [47, 48].

RANTES (regulated upon activation, normal T cell expressed) is a member of the CC chemokine family and is secreted by a variety of cell types, including T cells. The upregulation of RANTES has been observed in the mouse kidney following warm ischemia-reflow [6], and the inhibition of RANTES using Met-RANTES which is human RANTES with the addition of a single methionine residue, has also been reported to suppress the early migration of mononuclear cells into kidney in a cold ischemia-reflow model [49].

Mycophenolate mofetil (MMF), a prodrug type inosine monophosphate dehydrogenase (IMPDH) inhibitor, is de esterified to the active form, mycophenolic acid. IMPDH is the rate-limiting enzyme in the de novo guanosine nucleotide synthesis pathway. T and B cells are more dependent on this pathway than other cells, resulting in the specific anti-proliferative effect of MMF on T and B cells. Ysebaert et al. [50] have tested this drug for therapeutic potential against ischemic ATN using rat warm renal

ischemia-reflow model. MMF induced almost complete arrest of the T cell proliferation after ischemia.

FTY720 acts as a sphingosine 1-phosphate receptor-1 agonist, inducing its receptor internalization, and causes sequestration of circulating lymphocytes to secondary lymph tissue compartments. FTY720 does not impair the proliferation of T and B cells. A protective effect of FTY720 has been reported in murine warm renal ischemia reflow models [51, 52]. However, it is possible that FTY720 effect occurs independently of T cells through some yet to be determined mechanisms.

Statins, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors, lower plasma cholesterol concentration by mechanisms through which the statins inhibit the synthesis of cholesterol and increase the expression of LDL receptors in liver. Other than a cholesterol lowering effect, statins decrease the recruitment of T cells into the arterial wall and inhibit T cell proliferation and activation, suggesting an immunomodulatory effect of statins. This immunomodulatory effect of statins may be beneficial in ARF patients. So far three groups have evaluated the effects of statins on experimental ischemic ATN [53-56]. All these data suggest that statins improve the course of warm renal ischemic-reflow injury in mouse and rat. Statins may also be protective in other forms of ARF, such as preventing contrast-induced ARF [57].

Conclusions

Several decades of research have elucidated major mechanisms underlying ischemic ATN, but translational efforts in humans have yielded disappointing results. The limitations in our understanding of human ischemic ATN have led to close scrutiny of existing models for ischemic ATN. Future progress of an in silico model including comparison between human and animal ischemic ATN is promising. Furthermore,

recent recognition of the role of inflammatory cells such as T and B cells in ischemic ATN is now established area of research. Based on this recognition and feasible T cell therapeutic agents in man, effective therapies may well be forthcoming.

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Table 1. Comparison between in vitro, in vivo, and in silico models.

	In vitro models	*In vivo* models	*In silico* models
Pros	Reduced complexity of system	Similar complexity to human system Availability of transgenic and knock-out animals	Availability of human system information Analysis of thousands of genes and proteins at a time Comparison with any animal data base at any time
Cons	Physiological differences between *in vitro* models and *in vivo* counterparts The influences of surrounding cells	The animal models of ATN do not exactly mimic all the features of the human ischemic ATN Ethical consideration	Expensive Scanty data base
Best use of model	Simplistic characterization of ischemic ATN Screening for thousands of agents Evaluating the safety of drugs at a cellular level	Characterization of pathophysiological mechanism of ischemic ATN in a complex condition Discovery of therapeutic interventions Evaluating the safety of drugs	Characterization of molecular mechanisms of ischemic ATN Discovery of therapeutic interventions Evaluating the safety of drugs Prevision of future symptoms Diagnosis for diseases (ischemic ATN)
How to get access to the model	Literature Contacting the originators	Literature Contacting the originators	Literature Contacting the originators Web sites
Relevant patent	n/a	n/a	Web sites
References	[13-22] Related articles	[23-29] Related articles	[30-39] Related articles Web sites

n/a: not available

Table 2. T and B cell reagents recently identified to alter the course of experimental ischemic ATN.

Agent	ATN model	Time of treatment	Effect	Ref.
IL-10	mouse cisplatin	after	↓ Cr, ↓ TNF-α	42
IL-10	mouse warm ischemia-reflow	during	↓ Cr, ↓ TNF-α	42
IL-10	rat cold ischemia-reflow	after	↓ Cr	42
Anti-IL-12 antibody, IL-10	mouse warm ischemia-reflow	before	↓ TNF-α (Anti-IL-12 antibody, IL-10)	43
Anti-IL-6 antibody	mouse warm ischemia-reflow	before	↓ Cr, ↓ plasma Urea, ↓ TNF-α (only in IL-$6^{-/-}$mice), ↓ IL-1β (only in IL-$6^{-/-}$mice)	44
Bimosiamose	rat warm ischemia-reflow	before or after	↓ Cr (before) or No change in Cr (after), No change in $CD4^+$ T cell	45
Bimosiamose	rat cold ischemia-reflow	during (isograft) or after (allograft)	↑ GFR (during), ↓ Cr (during), ↓ SerumUN (during), ↓ TNF-α (after), ↓$CD4^+$ T cell (after), ↑ survival days (after), synergistic effect with FTY720 (after)	46
CTLA-4 Ig	rat cold ischemia-reflow	during and after	↓ Cr, ↓ TNF-α, ↓ IL-1β, ↓$CD4^+$ T cell	47
CTLA-4 Ig	rat warm ischemia-reflow	after	↓ Cr, ↓$CD43^+$ T cell	48
a combination of anti-B7-1 and -B7-2 antibody	rat warm ischemia-reflow	after	↓ Cr, ↓$CD43^+$ T cell	48
Met-RANTES	rat cold ischemia-reflow	after	No change in Cr, ↓$CD5^+$ T cell, ↓ TNF-α, ↓ IL-1β	49
Mycophenolate mofetil (MMF)	rat warm ischemia-reflow	after	No change in Cr, ↓ $CD4^+$ T cell	50
FTY720	mouse and rat warm ischemia-reflow	during	↓ Cr	51,52
cerivastatin	rat warm ischemia-reflow	before	↑ GFR, ↓ Cr	53
cerivastatin	mouse warm ischemia-reflow	before	↓ Cr	54
atrovastatin	rat warm ischemia-reflow	before	↑ GFR	55
pravastatin	mouse warm renal ischemia-reflow	Before	↓ Cr	56

Cr, serum or plasma creatinine; GFR, glomerular filtration rate; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; IL-12, interleukin-12; SerumUN, serum urea nitrogen; TNF-α; tumor necrosis factor-α.

Figure legend

Figure 1. Ischemia acute tubular injury and inflammatory cells

Initially ischemia causes injury of endothelial cells, followed by leukocyte activation and formation of platelet-leukocyte plugs. Chemokines and cytokines produced by both leukocytes and tubular cells lead to the recruitment of inflammatory cells from the microvasculature to the interstitium, allowing inflammatory cells to be able to interact with tubular epithelial cells. Renal inflammation is associated with the shortened microvilli of tubular epithelial cells and to the denuded epithelium. The sloughed cells adhere to each other and in turn form intratubule casts. Abbreviations: B, B cells; ICAM-1, intercellular adhesion molecule-1; T, $CD4^+$ T cells; Th1, T helper1 cells; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

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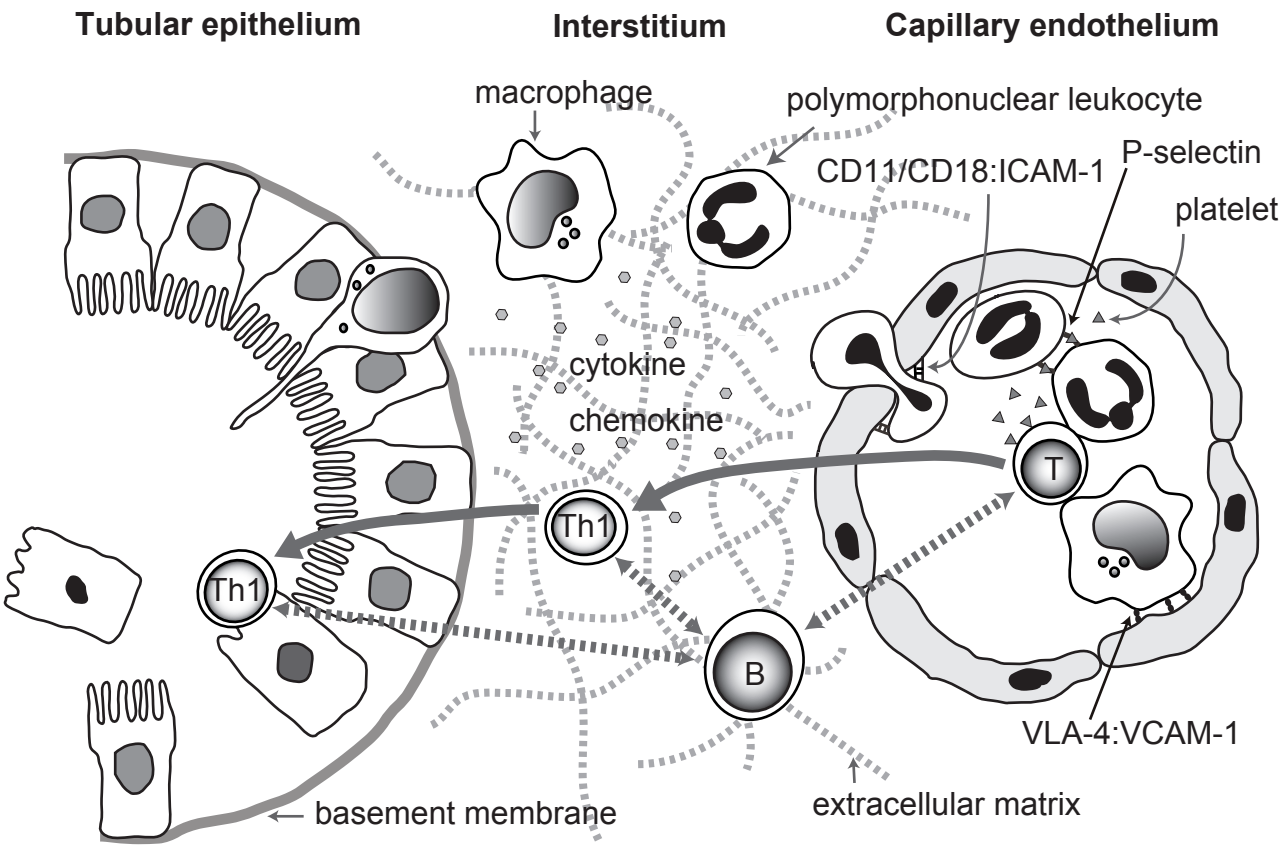

Figure 1. Ischemia acute tubular injury and inflammatory cells.

Initially ischemia causes injury of endothelial cells, followed by leukocyte activation and formation of platelet-leukocyte plugs. Chemokines and cytokines produced by both leukocytes and tubular cells lead to the recruitment of inflammatory cells from the microvasculature to the interstitum, allowing inflammatory cells to be able to interact with tubular epithelial cells. Renal inflammation is related to the shortened microvilli of tubular epithelial cells and to the denuded epithelium. The sloughed cells adhere to each other and in turn form intratubule casts. Abbreviations: B, B cells; ICAM-1, intercellular adhesion molecule-1; T, CD4+ T cells; Th1, T helper1 cells; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.