J Mol Med (2004) 82:91–101
DOI 10.1007/s00109-003-0498-8

REVIEW

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Leukocyte recruitment and acute renal failure

Received: 1 August 2003 / Accepted: 22 September 2003 / Published online: 10 December 2003

Abstract Despite advances in medical technology, acute renal failure (ARF) still represents a major challenge in clinical medicine, as morbidity and mortality have remained unchanged over the past two decades. The pathophysiology of ARF is highly complex and only poorly understood; new insights into the pathophysiology of ARF are therefore of utmost importance to develop better understanding and therapies. Acute tubular necrosis (ATN) is the predominant cause of ARF and often arises as a consequence of septic, toxic, or ischemic insults. The recruitment of leukocytes into the kidney has recently emerged as a key event in the development of experimental ischemic and septic ARF. A few descriptive clinical studies support this idea. However, the clinical relevance of various animal models remains unclear, as does the importance of different leukocyte subsets, and even methodological aspects as how to quantify renal leukocyte recruitment. This review summarizes and critically evaluates experimental findings that provide insight into the role of leukocytes and their recruitment during ARF. We aim to provide a valid description of ARF, illustrate animal models of ARF, review qualitative and quantitative methods to assess renal leukocyte recruitment, and discuss the components of the leukocyte recruitment cascade and their role in ARF.

Keywords Kidney failure, acute · Leukocytes · Neutrophils

Abbreviations *ARF:* Acute renal failure · *ATN:* Acute tubular necrosis · *ICAM:* Intercellular adhesion molecule · *I-R:* Ischemia-reperfusion · *LPS:* Lipopolysaccharide · *MPO:* Myeloperoxidase · *MSH:* Melanocyte-stimulating hormone · *PAF:* Platelet-activating factor · *PMN:* Neutrophil

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Introduction

Acute renal failure (ARF) is characterized by a sudden decrease in kidney function which leads to the inability to excrete metabolic waste and to maintain fluid as well as electrolyte homeostasis [1]. ARF continues to be a major clinical challenge as mortality and morbidity have not changed over the past two decades. Depending on the

severity, the mortality rate varies between 20% (including mild forms) and 70% (ARF in critically ill patients requiring renal replacement therapy) [2, 3].

According to the origin of the insult, ARF can be classified as (a) prerenal, (b) intrarenal, or (c) postrenal. Prerenal ARF is characterized by a decreased, insufficient renal perfusion that often occurs due to intravascular volume depletion, systemic hypotension, reduced cardiac output, or hepatorenal syndrome (renal insufficiency due to end-stage liver disease). When structures within the kidney such as glomeruli, tubules, vessels, and interstitial tissue are affected, ARF is called intrarenal. Acute tubular necrosis (ATN) is the leading cause of intrarenal ARF [3] and is often induced by ischemia or toxins. Prerenal ARF frequently precedes intrarenal ARF, since uncorrected, insufficient renal perfusion ultimately leads to ATN due to ischemia. Taken together, prerenal and intrarenal forms account for approximately 75% of cases of ARF [2]. Postrenal ARF usually indicates bilateral hindrance of urinary flow anywhere from the renal pelvis to the urethra and accounts for roughly 5% of cases in the hospital [2].

Therapy for ARF is limited to reversal of underlying causes (correction of hypovolemia or cardiac dysfunction) and to renal replacement therapies, which can only substitute for the excretory function of the kidney. Prevention and especially a better knowledge of renal pathophysiology are therefore most important to overcome the high morbidity and mortality associated with ARF.

In recent years many experimental studies, in particular renal ischemia-reperfusion studies, have led to the idea that ARF is mediated largely by an inflammatory reaction in response to the original insult. The inflammatory reaction includes activation and recruitment of various leukocytes, initiation of coagulation pathways, endothelial cell activation, and generation of reactive oxygen species and destructive enzymes (for further reference see [4, 5]). A few available clinical studies support this idea [6, 7, 8, 9]. Tissue edema, congested microcirculation, and detachment of tubular epithelial cells are typical morphological findings in severe experimental ARF.

Numerous studies have analyzed the recruitment of leukocytes and its underlying mechanisms during ARF. Despite several experimental studies that have clearly demonstrated a key role for leukocyte recruitment in the development of ARF, there are still controversies that surround the concept of leukocyte mediated ARF. Clinical relevance of animal models, types of leukocytes involved, and methods to quantify leukocyte recruitment are part of these controversies.

This review focuses on discussing experimental data that allow a mechanistic evaluation of leukocyte-mediated ARF. Particular emphasis is given to animal models of ARF, to qualitative and quantitative methods for assessment of renal leukocyte recruitment, to mechanisms and mediators of leukocyte recruitment and their role in ARF, and to potential future directions.

Animal models of ARF

As with many other experimental models of acute diseases, animal models of ARF usually do not consider the fact that under clinical circumstances ARF arises in the setting of preexisting renal diseases and/or multiple renal insults. Animal in vivo models of ARF can nonetheless mimic many, though not all, (systemic) aspects of human ARF. Table 1 provides a summary and comparison of currently employed animal models to study in vivo ARF.

Ischemia-reperfusion models

Ischemia-reperfusion (I-R) models are by far the most widely employed and discussed models for leukocyte recruitment during ARF (e.g., [10, 11]). Here, the renal vascular pedicle or the renal artery alone are clamped for various amounts of time leading to complete cessation of blood flow (ischemia); reperfusion is initiated by clamp removal. Ischemia is the basic insult, followed by an inflammatory reaction during subsequent reperfusion. The advantages of these models include (a) simplicity and reproducibility, (b) ease of injury control by varying duration of ischemia, and (c) inflammatory response similar to that seen in humans. However, one common major disadvantage of these models is the fact that ARF due to pure ischemia is unusual in clinical practice, as a complete stop of renal perfusion is only seen under special circumstances, for example, embolism of the renal artery or suprarenal aortic cross-clamping during vascular

Table 1 Animal models of experimental acute renal failure: currently employed animal models of experimental acute renal failure with respect to experimental difficulty (+ low, +++ high), ability to manipulate the actual insult (+ limited, +++ excellent), current knowledge about the model (+ scant, +++ rich), and clinical relevance (+ low, +++ high)

	Ischemia-reperfusion	LPS injection/ infusion	Infusion/injection of bacteria	Cecal ligation and puncture	Cardiac arrest (global ischemia)
Experimental difficulty	+	+	++	++	+++
Manipulation of insult	+++	+++	+++	++	+
Current knowledge	+++	++	++	++	+
Clinical relevance	+	+	++	+++	+++

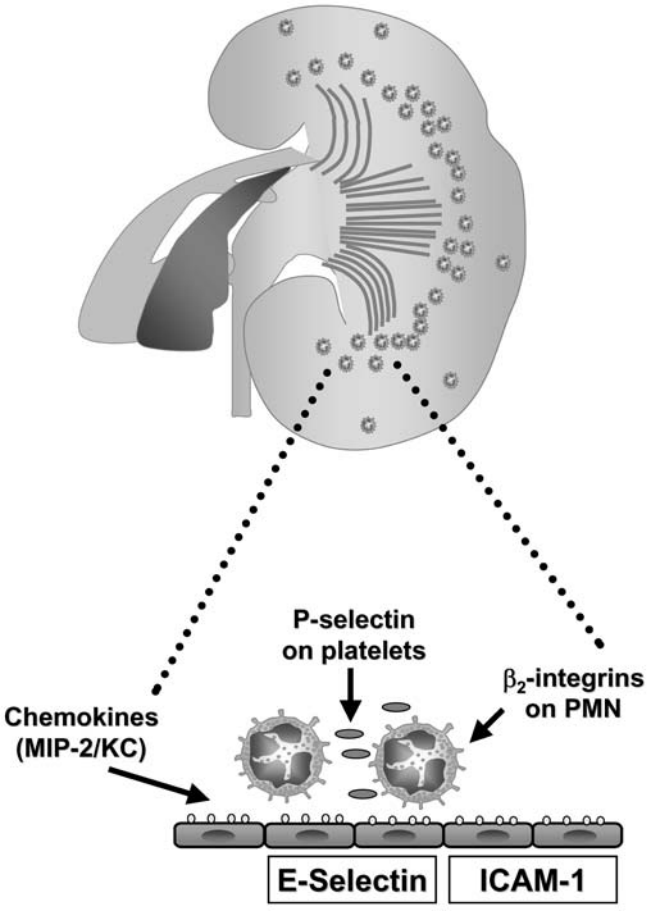

Fig. 1 Localization and mediators of PMN recruitment in postischemic ARF. Renal ischemia-reperfusion leads to endothelial activation and subsequent recruitment of PMN. Renal PMN recruitment occurs mainly in the outer medulla and to a lesser extent in the cortex (*upper panel*). Endothelial cell adhesion molecules (e.g., E-selectin, ICAM-1), CXC chemokines (KC/MIP-2), β_2-integrins on PMN, and platelets (via P-selectin) have so far been shown to play a key role in the development of experimental postischemic ARF

surgery. Several studies demonstrated that postischemic neutrophil (PMN) recruitment predominantly occurs in the outer medulla [12, 13, 14, 15] and to a lesser extent in the cortex [16] (Fig. 1).

Cold ischemia–warm reperfusion is a special form of I-R that is only infrequently used to study ARF and leukocyte recruitment [17]. The kidney is removed, flushed, stored at low temperature for different time periods, and implanted thereafter to mimic organ preservation and subsequent cadaveric transplantation. Renal inflammation induced by cold ischemia-warm reperfusion can differ from that observed in warm I-R [17]. In addition to a lack of widespread use, this model also is experimentally difficult, as it requires multiple microsurgical anastomoses.

Sepsis models I: endotoxin infusion or injection

This model is characterized by single injections (intravenous or intraperitoneal) or continuous infusion of bacterial lipopolysaccharide (LPS) and eventually subsequent fluid resuscitation (e.g., [18, 19]). In addition to affecting many other organs, for instance, lung and liver, systemic LPS administration leads to intrarenal vasoconstriction, reduced glomerular filtration rate, activation of coagulation-fibrinolysis cascade, and recruitment of leukocytes [20, 21]. The advantages of these models are simplicity and well-controlled insult. On the other hand, experiments might be flawed by inadequate fluid resuscitation [22] or by different lots of LPS with variable biological effects. Experiments are also hard to compare when the routes of LPS administration are not identical [23].

Sepsis models II: bacterial infusion

These models have also been performed in small and large animals by intravenous infusion of live bacteria. The pathophysiological characteristics are comparable to that seen with LPS administration. In addition to advantages and disadvantages seen with LPS administration, bacterial infusions lead to systemic hemodynamic responses comparable to human sepsis [24]. These models require standard supportive measures such as antibiotics or appropriate fluid resuscitation that are often not included or reported; therefore evaluation of results from different studies is often difficult.

Sepsis III: cecal ligation and puncture

Through laparotomy, the cecum is exposed and punctured with a needle, which permits colonization of the peritoneal cavity by gastrointestinal bacteria and subsequent peritonitis [25]. This mimics the human situation with an ongoing infection. These models are also rather simple and inexpensive, and they can be performed in both small and large animals. On the other hand, ATN cannot always be reproduced, the necessary supportive therapy (e.g., antibiotics, fluids) is not standardized, and variations may influence the study outcome.

Cardiac arrest as a model of whole-body ischemia to induce ARF

This very recent model [26] of experimentally induced ARF in mice is based on KCl-provoked cardiac arrest with delayed resuscitation (ventilation, chest compression, and epinephrine administration). A transient, approximately threefold increase in serum creatinine concentration characterizes this model of ARF. As opposed to the isolated renal I-R model, this model intends to mimic a far more common clinical situation,

i.e., whole-body ischemia due to cardiac arrest; it is therefore of high clinical relevance.

In all these models, leukocyte recruitment is part of the inflammatory response to a harmful insult. To demonstrate the functional relevance of leukocyte recruitment for ARF, the impact of the actual insult and that of the inflammatory response must be assessed carefully. The actual insult must be severe enough to cause a profound inflammatory reaction. However, if the insult is too strong or lasts too long, the inflammatory reaction might occur but has no functional consequence, as the tissue has already been lethally damaged. If the period of ischemia is, for example, too long, the tissue becomes (vastly) necrotic even before reperfusion starts. On the other hand, an insult that is too weak to cause a substantial inflammatory response may not induce ARF. Consequently, negative findings regarding the impact of inflammatory leukocyte recruitment on tissue damage or organ dysfunction must be interpreted with careful attention to the intensity of the actual insult.

Qualitative and quantitative methods to assess leukocyte recruitment

To thoroughly explore the role of leukocytes in ARF, the renal recruitment of leukocytes and their subsets and the functional impact of these cells must be quantified. So far, the overwhelming majority of studies have exclusively looked at PMN as the most important and prevalent leukocytes during ARF. Several techniques that aim to qualitatively or quantitatively analyze PMN recruitment have been published.

Qualitative assessment of PMN recruitment: the role of PMN in experimental ARF

Ideally, the role of PMN should be explored by incorporating "PMN-depletion" techniques into experimental models of ARF. This approach can use a variety of strategies to deplete the blood from circulating PMN prior to experimental induction of ARF: polyclonal anti-PMN serum [10, 11], cytostatics such as hydroxyurea [27] and nitrogen mustard [28], or monoclonal antibodies against PMN [29]. To interpret the findings from such interventions correctly, the (potential) side effects of each technique must be considered. The application of heterologous anti-PMN serum warrants close monitoring of all leukocyte counts since anti-PMN sera can also affect monocytes [30]. The inclusion of proper control experiments (e.g., injection of preimmune serum into another group of animals) and careful monitoring of differential leukocyte counts can help to overcome these problems and make anti-PMN serum an inexpensive and valuable tool to study PMN-dependent ARF [10, 11]. Studies that use cytostatics such as nitrogen mustard and hydroxyurea should be interpreted carefully, as cytostatics are known to deplete not only PMN but also monocytes, lymphocytes, and even platelets. Experiments with monoclonal anti-PMN antibodies also require appropriate controls and differential blood counts, because some monoclonal antibodies can also bind to monocytes/macrophages [31].

Carefully performed and well-controlled PMN-depletion studies with respect to experimental ARF have so far been conducted only for I-R induced ARF [10, 11, 32]. These experiments unequivocally demonstrate a key role for PMN in the development of ARF since neutrophil-depleted mice were almost completely protected from postischemic ARF. Moreover, renal dysfunction (serum creatinine concentration) depends directly on circulating PMN count at the time of ischemia [10].

Quantitative assessment of PMN recruitment

Renal PMN recruitment can be quantified using the myeloperoxidase (MPO) assay. MPO is a granular enzyme found in both PMN and monocytes/macrophages. However, the MPO concentration in monocytes is at least three times lower than that in PMN [33, 34]. These findings have made MPO assays a widely accepted technique to quantify tissue PMN content. However, although the MPO concentration in PMN is substantially higher than that in monocytes/macrophages, the contribution of the latter to inflammatory tissue injury is still possible. Additionally, prolonged increases in renal MPO activity may not only result from the infiltration of just PMN but also reflect a later/delayed infiltration of monocytes/macrophages. These problems can be overcome by quantitative immunohistological analyses. Although morphometric studies are extremely labor intensive and time consuming, renal MPO activity may be compared between untreated and PMN-depleted mice during ARF. In a recent renal I-R study [10] the increase in renal MPO activity at 24 h after I-R was entirely due to increased PMN recruitment. Compared to mice injected with preimmune serum (control), mice that had received a polyclonal anti-PMN serum revealed renal MPO activity after I-R that was as low as those observed in sham-operated mice. Except for the intended neutropenia, the differential leukocyte counts in PMN-depleted mice remained normal. Therefore renal MPO activity can serve as a valid marker of renal PMN infiltration, if the experimental model is well defined and controlled. Without differential leukocyte counts, renal MPO data must be interpreted carefully and should only be used as an indicator of global leukocyte infiltration [35].

Some technical aspects also warrant special attention when performing renal MPO assays. Kidney tissue contains inhibitors of MPO activity [33, 36, 37]. These inhibitors can be inactivated by two procedures [36, 38]: intense, repeated washing immediately after tissue homogenization or addition of chelating agents to the MPO extraction buffer. A combination of these two techniques to measure renal MPO activity [10, 11] is superior to the incubation of kidney specimen at 56–60°C for 2 h, as previously suggested [39].

Table 2 Role of leukocytes and mediators of their recruitment in experimental models of acute renal failure: current knowledge regarding the role of leukocytes and mediators of their recruitment in experimental acute renal failure (++ critical role, strong and clear experimental evidence, + critical role, but requires further experimental elaboration, – experimental data suggest no role, *?* role is unknown, no experimental data available, *Lym* lymphocytes)

	Leukocytes		Selectins			Integrins (incl. ligands)		Chemoattractants	
	PMN	Lym	E	L	P	CD11a/b	ICAM-1	Classical	Chemokines
Ischemia-reperfusion	++	++	++	–	++	++	++	+	+
Sepsis	+	?	+	?	+	?	?	?	?
Whole-body ischemia	?	+	?	?	?	?	?	?	?

Another very popular method to quantify renal PMN recruitment is morphometric (immuno-)histology. Among histochemical stains, the naphtol AS-D-chloroacetate stain (Leder's stain) is considered more sensitive for PMN than regular hematoxylin-eosin stain, as it yields brilliant red staining of PMN in the absence of staining of other cells. The specificity of both stains, however, has been questioned. Different fixation techniques could at least partially explain the controversies, since fixation with 10% formalin significantly reduces the naphtol AS-D-chloroacetate staining intensity of neutrophils [13]. For immunohistochemistry, several authors have successfully used anti-PMN monoclonal antibodies in both mice [14, 15] and rats [40] to identify PMN in renal tissue; nonetheless, concerns have arisen with respect to the specificity of the rat monoclonal antibody that can bind to monocytes/macrophages as well [31]. As noted above for PMN depletion experiments or MPO assays, the problems regarding the specificity can be overcome by confirmation of the specificity for PMN staining in peripheral blood smears [13].

Qualitative and quantitative assessment of leukocytes other than PMN

Compared to the plethora of data regarding PMN recruitment and ARF, the recruitment of other leukocytes, such as lymphocytes or monocytes, has not been investigated until recently. Motivated by studies that found lymphocyte-associated cytokines during experimental ARF or evidence of lymphocyte-mediated tissue injury in other organs [41, 42], a few studies have recently reported evidence for a role of leukocytes other than PMN in experimental ARF [26, 43, 44] (Table 2).

Techniques to investigate the qualitative impact of lymphocytes on experimental ARF have so far been restricted to gene-deficient mice. Interestingly, mice that are deficient in T lymphocytes (*nu/nu* mice [44]) or in $CD4^+$ T lymphocytes [43] exhibited a significant protection from I-R induced ARF, which was accompanied by diminished renal PMN-recruitment. In a model of whole-body ischemia (cardiac arrest), *nu/nu* mice also demonstrated significantly smaller rises in serum creatinine concentrations and lower renal injury scores, whereas renal MPO did not change [26]. The relationship between T cells and PMN recruitment remains to be explored.

Mice that lack both B and T lymphocytes (RAG-1 deficient mice) did not display any protection in an I-R model of ARF [45]. Although upregulated natural killer cell activity in RAG-1 deficient mice [46] may help to explain the surprising contradiction, this finding emphasizes the need for thorough and conclusive control experiments. Reconstitution experiments are required to distinguish between "gene-deficiency effects" and compensatory pathways that may have developed. At least one of the above studies included appropriate control experiments [43], supporting a role for T lymphocytes in experimental ARF.

Quantitative measurements of renal lymphocyte recruitment are limited to (immuno-)histochemistry and morphometry, as there are no known enzymatic or biochemical markers that are specific for lymphocytes. These techniques, however, are also subject to the caveats noted above, for example, antibody specificity. Flow cytometry is another technique to study renal leukocyte populations [47] but has not been employed in experimental ARF yet.

Contrast ultrasound is a rather recently developed technique that permits quantitative, noninvasive measurements of renal leukocyte recruitment in vivo. Here, ultrasound contrast agents consisting of gas-filled microbubbles (lipid or albumin shell, approx. 4–6 μm in size) target inflamed tissue due to their adherence to and subsequent phagocytosis by PMN and monocytes [48]; these effects are either by β_2-integrin- (albumin shells) or by complement-mediated (lipid shells). Phagocytosed microbubbles keep their acoustic properties and are therefore detectable by ultrasound. Approximately 10 min after a bolus injection, all freely flowing microbubbles will have been washed out, and adherent microbubbles will have been completely phagocytosed [49]. Using ultrasound imaging with background-subtracted video intensity technique, one can display the amount and localization of phagocytosed microbubbles within the inflamed tissue, which allows spatial and quantitative resolution of leukocyte recruitment (only PMN and monocytes) into this tissue [49]. This strategy has so far been used in a previously published model or renal I-R [10, 11]. In this study, the ultrasound signal intensity correlated well with renal MPO activity [14].

Leukocyte recruitment into inflamed tissue

Leukocyte recruitment into most organs occurs in a cascadelike fashion that encompasses capture, rolling, activation, firm adhesion, and transmigration of leukocytes [50, 51, 52]. Capture and (slow) rolling are largely mediated by the selectin family of molecules, which consists of three molecules: E-, L-, and P-selectin [53]. Selectins are type I cell-surface glycoproteins and contain an N-terminal domain homologous to lectins, which can interact with sialylated motifs on mucinlike glycoproteins. L-selectin is expressed on all monocytes and granulocytes as well as on most lymphocytes. P-selectin is stored in α-granules of platelets and in Weibel-Palade bodies of endothelial cells; upon activation it can rapidly be brought to the cell surface. Except for skin microvessles, E-selectin is not expressed under baseline conditions but can be quickly synthesized following cytokine activation of endothelial cells. Although several molecules have emerged as potential selectin ligands, only P-selectin glycoprotein ligand 1 has been clearly shown to act as a functional ligand for all three selectins [54, 55, 56].

Rolling leukocytes can be activated by chemoattractants. Chemoattractants compromise a wide group of molecules that activate heptahelical, G-protein-coupled receptors [51]. Whereas classical chemoattractants, such as lipid mediators (e.g., platelet-activating factor), complement components (e.g., C5a), and formylated bacterial peptides (e.g., formyl-methionyl-leucyl-phenylalanine), can attract/activate most leukocytes, members of the chemoattractive cytokine (chemokine) family exhibit a rather selective capability to attract/activate only certain subsets of leukocytes [57, 58, 59]. Chemokines can be classified according to the position of their N-terminal cysteine residue into C-, CC-, CXC-, and CX_3-chemokines. The presence of a so-called ELR-motif (glutamic acid-leucine-arginine) in the N-terminal region of CXC-chemokines allows further classification of these chemokines into ELR positive and ELR negative chemokines; ELR^+ chemokines are known to activate PMN.

Upon activation, leukocyte integrins change their confirmation and bind to their ligands to promote firm adhesion. Integrins are $\alpha\beta$-heterodimeric membrane proteins [60, 61]. Integrin activation mediated by inside-out signaling is required for binding to their immunoglobulin (Ig superfamily) and extracellular matrix protein ligands. For PMN recruitment, β_2-integrins (CD18) are most important [62, 63].

Transendothelial migration (TEM) is the last step of leukocyte recruitment into inflamed tissue [64, 65]. TEM occurs by leukocyte diapedesis through lateral adherens junctions at tightly associated endothelial cells [66]. Although previous results suggest that neutrophils can circumvent the junctional barrier and transmigrate through preexisting discontinuities at tricellular corners [67], the reversible remodeling of adherens junctions appears to be the key event in leukocyte TEM [68]. Several molecules have so far been implicated in leukocyte TEM: β_1-integrins [69, 70], platelet cell adhesion molecule (member of the Ig superfamily) [71, 72], junctional adhesion molecule-1 (member of the Ig superfamily) [73, 74], and CD99 [75].

Although the recruitment of leukocytes into inflamed tissue and its mediators is usually proposed as a cascadelike sequence of events, recent data demonstrate that the mediators involved share overlapping functions and are not strictly confined to only one aspect, for example, selectins can mediate both rolling and firm adhesion, whereas integrins can engage slow rolling and firm adhesion [76].

Mediators of leukocyte recruitment in experimental ARF

As outlined below, several mediators of leukocyte recruitment and their impact on the development of experimental ARF have been investigated to date. The vast majority of studies have dealt with models of postischemic ARF (I-R-induced ARF).

Selectins

Studies in gene-deficient mice have demonstrated a key role for E- and P-selectin in I-R-induced ARF [10, 11], as these mice had at least a 80% protection from ARF, presumably due to reduced PMN-recruitment. In this strongly neutrophil-dependent model of severe ARF (see above), I-R consisted of 32 min ischemia followed by reperfusion. A previous study had shown a 50% mortality rate under these circumstances [32]. The protective effect of E- or P-selectin blockade has also been demonstrated by injection of blocking monoclonal antibodies [10, 11]. These data, together with the observation that mice gene deficient in both E- and P-selectin reveal no further protection (unpublished data), lead to the conclusion that both E- and P-selectin are necessary but not sufficient to mediate ischemic ARF. Using the same model of ARF along with bone marrow transplantation, a subsequent study showed that platelet P-selectin, and not endothelial P-selectin, is the key component in P-selectin mediated ARF [15]. Two mechanisms could explain these effects: (a) platelets may adhere to the endothelium and subsequently allow leukocytes to adhere, or (b) circulating platelets could adhere to neutrophils and then form aggregates which become trapped in narrow peritubular capillaries. There is experimental support for both hypotheses. Several in vitro and in vivo studies, in particular angioplasty and atherosclerosis studies [77, 78, 79, 80, 81], have clearly shown that (activated) platelets actually recruit leukocytes to sites of inflammation. On the other hand, an intravital microscopy study of intestinal I-R explicitly showed that the blockade of endothelial P selectin completely abolished platelet-endothelial cell interactions whereas platelet-leukocyte interactions were strongly dependent on platelet P-selectin [82]. In a similar

but less severe model of I-R induced ARF (i.e., only transient increase in serum creatinine over 48 h after 30 min of ischemia), L-selectin gene-deficient mice did not reveal any protection from ARF [83].

The concept of selectin blockade to reduce PMN recruitment into postischemic kidneys and thereby protect from ARF gains further support by two recently published studies. Pharmacological inhibition of all three selectins and gene deficiency in fucosyltransferases IV and/or VIII, which eliminates functionally important fucose groups to selectin ligands, resulted in a significant attenuation of postischemic PMN recruitment as well as in protection from ARF after single kidney ischemia (for 30/45 min in rats plus contralateral nephrectomy) and after bilateral ischemia (30 min in mice), respectively [84, 85]. Gene deficiency in fucosyltransferase VII alone was more protective than that in fucosyltransferase IV [85].

In a "cecal ligation and puncture" model of septic prerenal azotemia, i.e., prerenal ARF (increase in blood urea nitrogen >increase in serum creatinine concentration), mice gene-deficient for E-selectin, P-selectin, or both were completely protected [86]. Selectin-deficient mice revealed unchanged intraperitoneal leukocyte recruitment but altered cytokine levels when compared to wild-type mice. The authors therefore concluded that selectins exert their effects through modulation of systemic cytokine profiles rather than through engagement in leukocyte-endothelial cell interactions.

Treatment with sialyl Lewis^x, a selectin ligand, in rabbits also resulted in an (almost) complete protection from ARF at 6 h after LPS infusion [18]. This protection was associated with a decrease in renal PMN-recruitment as assessed by morphometric histology.

Chemoattractants

In a rat model of I-R-induced ARF (30-min, bilateral clamping), the oral administration of a pharmacological platelet-activating factor (PAF) antagonist led to an approximately 30% protection from renal dysfunction 24 h after ischemia and to significantly diminished postischemic PMN recruitment (MPO assay) [87]. Anti-PAF treatment was effective if given either before or immediately after ischemia; delayed treatment with the PAF antagonist had no effect. Additional inhibition of intercellular adhesion molecule (ICAM) 1, a member of Ig superfamily and β_2-integrin ligand, by a monoclonal antibody did not confer any further protection. The results from this study suggest PAF-mediated PMN recruitment as a key component in I-R induced ARF.

Blockade of the CXC-chemokines MIP-2/KC by monoclonal antibodies in a murine model of I-R induced ARF (60 min of ischemia) led to an improved survival rate and an approximately 55% reduction in renal dysfunction, as measured by serum creatinine concentrations [88]. Postischemic PMN recruitment (histology) and renal tissue injury were also significantly lower, by 65% and 60%, respectively. PMN depletion prior to I-R with a monoclonal antibody revealed protective effects similar to those observed with antibody-administration; however, no differential leukocyte counts were given. This study indicates that chemokine-mediated PMN recruitment may be an important factor in I-R-induced ARF.

Lipoxins are endogenous lipoxygenase-derived eicosanoids that exhibit anti-inflammatory, vasodilatory, and pro-resolution properties. In a murine model of severe postischemic ARF, treatment with 15-epi-16-(*para*-fluorophenoxy)-lipoxin A(4)-methyl ester, a synthetic analogue of 15-epi-lipoxin A(4) greatly reduced ARF by about 70% [89]. In addition to significantly blunted renal PMN recruitment and better tissue injury scores, this treatment also caused decreased (mRNA) levels of interleukin 1β, interleukin 6, and KC but not of ICAM-1 or vascular cell adhesion molecule. Further in vitro experiments showed that lipoxin A4 can inhibit chemoattractant-induced PMN transmigration. These results demonstrate that lipoxin A4 agonists are powerful mediators in postischemic ARF. Because of their multiple sites of action and the results presented, modulation of PMN recruitment appears as one target of lipoxin A4 agonists, but other effects may also contribute to protection.

Integrins and their immunoglobulin (Ig)-like ligands

Integrins and their Ig-like ligands are among the best-studied mediators of leukocyte recruitment in the context of I-R induced ARF. ICAM-1, which is expressed on endothelial cells and many other cells, is a counterreceptor for the PMN β_2-integrins lymphocyte function antigen 1 (CD11a/CD18) and Mac-1 (CD11b/CD18).

Compared to a control group, antibody blockade of CD11a and CD11b in a rat model of renal I-R (single kidney ischemia for 60 min plus contralateral nephrectomy) resulted in approximately 30% lower peak serum creatinine concentrations 24 h after I-R; the histopathological damage score was also significantly reduced [90]. The potential of ICAM-1 inhibition as a preventive or therapeutic intervention in I-R induced ARF has been shown by several studies [32, 91, 92, 93]. Independently of species (rat or mouse) and of blocking strategy (antibodies, antisense nucleotides, and knock-out mice), all studies demonstrated a strong protective effect due to ICAM-1 blockade. This was always associated with significantly diminished postischemic PMN recruitment, as assessed by both histology and MPO assay, as well as with better tissue injury scores. Through the administration of anti-PMN serum and respective control serum, only one of these studies clearly demonstrated its particular model of I-R induced ARF to be PMN dependent [32]. Taken together, these data indicate a vital role for ICAM-1 in I-R mediated ARF. Although all studies also revealed a reduced postischemic PMN-infiltration, one cannot automatically assume that ICAM-1 acts mainly by the modulation of PMN recruitment, as renal ICAM-1 expression is not limited to

endothelial cells but can also be found in interstitial cells. Further studies are necessary to fully elaborate the impact of various sites of ICAM-1 expression.

Indirect mediators of leukocyte recruitment

In addition to the above mediators of renal leukocyte recruitment, there are also indirect modulators of PMN recruitment during ARF. The most prominent and best investigated among these are α-melanocyte-stimulating hormone (MSH) and adenosine receptor agonists. Previous studies have unequivocally demonstrated a strong protective effect of α-MSH in severe I-R induced ARF (mice and rats) [12, 94, 95]. These effects were accompanied by a significant reduction in PMN recruitment and by modulation of adhesion molecule and chemokine expression. All studies also showed other anti-inflammatory properties of α-MSH such as nitric oxide-synthase modulation. Moreover, one study also revealed significant effects of α-MSH even in ICAM-1 gene-deficient mice [94], suggesting that some of its effects may be PMN independent.

Adenosine A_{2A} receptor agonist can profoundly inhibit severe postischemic renal dysfunction in both mice (27–32 min bilateral ischemia) and rats (45 min of single kidney ischemia plus contralateral nephrectomy) [13, 94, 96]. In addition to a decreased renal PMN infiltration, probably due to diminished P-selectin and ICAM-1 expression, adenosine A_{2A} receptor agonists also seem to prevent the release of reactive oxygen species.

Conclusions and future directions

ARF is a common disease with a high overall mortality and therefore requires intense research efforts. Over the past decade, inflammation has come to be regarded as a major component in the development of (experimental) ARF. A plethora of data suggest leukocyte recruitment as a key event during the inflammatory response; here, especially the recruitment of PMN during postischemic ARF has been a major focus of research. Many experimental studies have shown protective effects when blocking mediators of leukocytes recruitment, such as adhesion molecules and chemoattractants. Controversies surrounding methodology and interpretation of these studies, however, have limited conclusions with respect to the overall concept of leukocyte-mediated ARF. Postischemic ARF is currently the only form of experimental ARF for which there is strong, explicit evidence that the recruitment of leukocytes, particularly PMN plays a causative role (see Fig. 1 for summary). Other forms of experimental ARF (e.g., septic ARF and ARF after global ischemia) seem to depend on certain leukocyte subsets and/or mediators of their recruitment. Currently, there is not sufficient information to conclude whether the effects are due to renal recruitment of these leukocytes or due to extrarenal events such as modulation of systemic cytokine profiles.

Future research may focus on two major aspects to clarify the concept of leukocyte-mediated ARF and to allow a successful translation into clinical studies. First, there is a critical need for carefully controlled animal models that incorporate both a high level of clinical relevance and appropriate techniques to study the recruitment of leukocytes and their subsets. Models of renal I-R mimic only a less common form of ARF and do not account for the complexity and the systemic interactions seen in more frequent forms of ARF such as septic or hypotensive/hemorrhagic ARF. The development of a model for ARF after global ischemia due to cardiac arrest represents an important step to solve this problem. The great impact of clinically relevant models must be accompanied by well-controlled and validated techniques to study leukocyte-mediated injury.

Second, future clinical studies should also explore the possibility that leukocyte recruitment into the kidney, especially during systemic inflammatory reactions, is only one form of leukocyte-mediated renal injury, and that leukocytes might induce renal damage from outside the kidney. Some leukocytes, for example, lymphocytes, are present in the kidney only in small amounts, even during the course of ARF but still seem to substantially contribute to renal dysfunction.

Research results obtained in the past decade hold promise for progress in the treatment of ARF, which hopefully will translate into lives saved and renal replacement therapies avoided.

Acknowledgements Our original work on ARF was supported by the Deutsche Forschungsgemeinschaft (DFG SI-680/1-1 to K.S.) and National Institutes of Health (NIH HL S4136 to K.L.).

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