Chapter

Mouse Models of Acute Kidney Injury

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Abstract

Acute Kidney Injury (AKI) is a poor prognosis in hospitalized patients that is associated with high degree of mortality. AKI is also a major risk factor for development of chronic kidney disease. Despite these serious complications associated with AKI there has not been a great amount of progress made over the last half-century. Here we have outlined and provided details on variety of mouse models of AKI. Some of the mouse models of AKI are renal pedicle clamping (ischemia reperfusion injury), Cisplatin induced nephrotoxicity, sepsis (LPS, cecal slurry, and cecal ligation and puncture), folic acid, and rhabdomyolysis. In this chapter we describe in detail the protocols that are used in our laboratories.

Keywords: ischemia reperfusion injury, cecal ligation and puncture, cecal slurry, sepsis, LPS, kidney, inflammation, immune cells, cisplatin, folic Acid, rhabdomyolysis, acute kidney injury, nephropathy

1. Introduction

Acute kidney injury (AKI) is a common clinical disorder characterized by a precipitous decline in renal function [1]. AKI is particularly prevalent in hospitalized patients and is associated with varied underlying etiologies, such as sepsis [2], cardiac surgery [3], rhabdomyolysis [4], and drug toxicity [5]. Patient outcomes are varied and depend partly on severity, with higher mortality seen in critically ill patients [1]. Importantly, patients who survive an episode of AKI are at increased risk for major adverse cardiovascular events, as well as for progression to chronic kidney disease (CKD) and end-stage renal disease (ESRD) [6]. Despite our growing understanding of the causes and mechanisms of AKI, as well as an effort to develop better diagnostic strategies, few preventive or therapeutic options exist.

Therefore, animal models of AKI are essential for identifying mechanisms of renal dysfunction and for development of therapeutic and diagnostic strategies [7]. To this end, mice have been the main experimental organisms for studying AKI. Since the underlying causes of AKI are varied, several murine animal models have been established. These animal models recapitulate several pathophysiological features of AKI, such as endothelial dysfunction [8], epithelial cell death [9], and immune cell infiltration [10] (**Figure 1**). In the current chapter, we provide an overview of the methods and highlight issues that are critical in establishing various murine models of AKI.

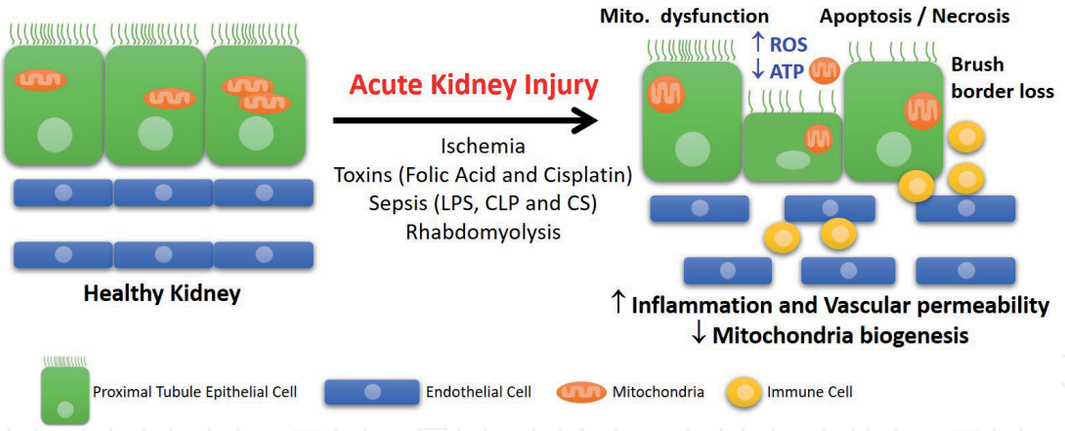

Figure 1.
Complex pathophysiology of AKI: Involvement of endothelium, renal tubules (specifically proximal tubule (PT) segment) and immune cells. Compared to normal/healthy kidney, various AKI insults result in overall decrease in ATP, loss of PT brush border, increase in vascular permeability and inflammation ultimately resulting in apoptosis and necrosis.

2. Ischemia reperfusion injury (IRI) model of acute kidney injury

2.1 Background

Mouse Model of Kidney Ischemia Reperfusion Injury: Warm ischemia and reperfusion injury models is the most widely used model of AKI, in rodents the renal blood flow is temporarily interrupted for a various time periods ranging from 20 to 45 minutes followed by varied amounts of reperfusion. In our laboratory we use a standard clamp time of 26 minutes followed by 24 hours of reperfusion. The IRI model of AKI can be further divided into bilateral, unilateral, and unilateral with simultaneous nephrectomy depending on the questions or therapeutic tested. In bilateral both renal pedicles are clamped, whereas unilateral with simultaneous nephrectomy can be utilized to reduce the variability that can be observed. However, in unilateral either left or right pedicle is clamped without disturbing the contralateral control kidney, ideally the unilateral IRI model is used for studies that have interest in evaluating progression to chronic kidney disease or fibrosis.

2.2 Methods

1. We routinely use 10–12 weeks old C57BL/6 male mice or either commercially purchased from vendor (Jackson Laboratories or NCI) or bred in house. The commercially purchased animals acclimate for one week in a 12-hour dark and 12-hour light cycle room prior to undergoing any surgeries. If using female or other strains (BALB/c or FVB) the ischemia time can be adjusted, ideally, we have established that 26-minutes of clamp time for C57BL/6 male mice and 28-minutes for BALB/c [11–13] or female C57BL/6 mice (unpublished observations) results in similar ischemic injury in our laboratory.

2. All surgical tools are sterilized by autoclaving before each experiment and between mice a hot bead sterilizer is used.

3. The mice are anesthetized with a mixture of Ketamine/Xylazine (120/12 mg/kg, Intraperitoneal injection) and as analgesic buprenorphine (0.15 mg/kg, subcutaneous injection) prior to placing on a warm pad (pad temperature is set to 34.6°C). Additionally, ophthalmic ointment is applied to the eyes following induction of anesthesia to prevent corneal drying.

4. The surgical site is shaved and cleaned by alternating between 70% ethanol and betadine (minimum 3 times).

5. Prior to any surgical procedure the mouse body temperature needs to reach 34°C (this will reduce overall variability in injury).

6. Bilateral flank incisions are made, and the renal pedicle (vein and artery) on both sides are clamped using micro-serrefine atraumatic vascular clamps (Fine Science Tools, 18055–02) for bilateral IRI. If mice are used for chronic studies, only clamp one side (we usually clamp the right pedicle as it is easier to perform nephrectomy on left contralateral control kidney 1 day before terminating studies, usually day 13) [14]. An alternative here can be to have surgical mice undergo simultaneous nephrectomy of contralateral control kidney. We do not practice this protocol in our lab as this could lead to a more severe kidney injury with standard clamp time of 26 minutes.

7. Sham mice will undergo the same procedure (steps 3-step 6) without applying the clamps to induce ischemia.

8. The mouse body temperature is checked intermittently during ischemia using rectal probe and maintained to be above 34°C along with visual confirmation of clamped kidneys (dark purple color).

9. The atraumatic clamps are removed and reperfusion on both sides is confirmed by checking the kidney color (changes from dark purple to pink). Both incision sites are closed with a 4.0 vicryl suture.

10. The mice recover from anesthesia on heating pad are returned to cages once awake and kept in a home-made chamber with a heating lamp to maintain chamber temperatures to around 30–33°C.

11. Depending on the protocol need for experiments, surgical mice will undergo reperfusion for 24–72 hours.

12. Kidney function can be rapidly assessed by measuring changes in plasma creatinine (Diazyme Laboratories) [11, 12, 14–16] and BUN. Histological changes are usually determined by analyzing hematoxylin and eosin (H&E) or periodic-acid Schiff (PAS) staining. All histology samples are scored for acute tubular necrosis (ATN). For quantification of tubular injury score, sections are assessed by counting the percentage of tubules that display cell necrosis, loss of brush border, cast formation, and tubule dilation as follows: 0 = normal; 1 = <10%; 2 = 10 to 25%; 3 = 26 to 50%; 4 = 51 to 75%; 5 = >75%. Five to 10 fields from each outer medulla are evaluated and scored in a blinded manner. The histological changes are expressed as ATN, scored as previously described [12, 17].

2.3 Critical notes

1. The IRI protocol in mice is not very technically challenging but does require some expertise. Some of the alternatives that can be used for a beginner is to do a midline laparotomy to have a better visual of the kidneys (step 6) or using flank incision gently pop each of the kidney out of the body cavity to have a better visual of the renal pedicle.

2. The clamping or ischemia times (20–45 minutes) may need to be changed if other anesthetic (pentobarbital sodium or isoflurane) is used.

3. The sex and age of mice also can contribute to the level of injury. Older mice and rats [18] are more susceptible to IRI induced AKI and it is also well-known and accepted that female [7, 19] mice are less susceptible compared to age matched male mice.

4. The type of mouse strain is also a critical variable that needs to be documented. Our recent unpublished work using C57BL/6 and DBA/2 J mice demonstrated that compared to C57BL/6 mice DBA/2 J mice are significantly protected from 26 minutes of bilateral IRI. Furthermore, these mice were also protected with cisplatin induce AKI. These preliminary observations from the Bajwa and Pabla labs indicate that strain of animals is a very important variable that needs to be considered in preclinical models of AKI.

5. In many of the studies all post-operative mice are supplemented with 0.5–1 ml of saline as a subcutaneous injection. This additional procedure can be applied depending on the protocols approved in your institution.

3. Cisplatin associated acute kidney injury

3.1 Background

Due to their role in the metabolism and excretion of xenobiotics, kidneys are particularly vulnerable to drug-induced toxicities [1]. The renal tubular epithelial cells have significant capacity for uptake of drugs, and this can result in high intracellular concentrations, which can lead to toxicities and development of acute kidney injury. Cisplatin is a widely used chemotherapy drug that accumulates in renal tubular cells causing acute kidney injury [5]. Cisplatin is used as part of chemotherapy regimens for the treatment of a wide spectrum of malignancies such as testicular, head and neck, ovarian, lung, cervical, and bladder cancers. Cisplatin accumulates in the tubular epithelial cells through organic cation [20] and copper transporters [21], which in turn activates a plethora of signaling pathways that culminate in epithelial cell death, inflammation, and kidney injury [5]. Tubulointerstitial injury is the predominant lesion observed during cisplatin nephrotoxicity, wherein both proximal and distal tubules are affected and display significant necrosis. It is observed that renal function improves in most patients, however a subset of patients can develop chronic renal impairment [22].

Cisplatin nephrotoxicity can be mimicked in murine models through single [16, 23] or multiple [24] injections. The single injection induced AKI is the most widely used model of cisplatin nephrotoxicity. In this model, a single intraperitoneal injection (10–30 mg/kg) results in development of AKI within 2–3 days.

3.2 Methods

1. Prepare a 1 mg/mL solution by dissolving cisplatin in normal saline (0.9% NaCl). To this end, add cisplatin to pre-warmed normal saline (37°C), followed by transferring the tube to a shaker at room temperature for 1 hour, along with intermittent vortexing. While this solution can be stored at room temperature (dark) and used for up to two weeks, preparing fresh solution on the day of injection generally leads to more consistent injury.

2. Inject cisplatin intraperitoneally using a 1 ml syringe with 26 G needle at a dose of 10–30 mg/kg body weight. We have found that dosing the mice in the

afternoon (2 PM) leads to more consistent injury and this might be related to the circadian regulation of organic cation transporters that are involved in renal uptake of cisplatin [25].

3. On day three post-cisplatin injection, sacrifice the mice by carbon dioxide euthanasia followed by serum and renal tissue collection for further analysis. Kidney injury can then be evaluated by serum analysis [blood urea nitrogen (BUN) and creatinine], histological analysis [hematoxylin–eosin (H&E) staining], and examination of renal expression of injury biomarkers [kidney injury molecule-1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL)] [26, 27]. Blood urine nitrogen and enzymatic assay-based creatinine measurements can be performed in serum or plasma samples using commercially available kits. For histological analysis, mouse kidneys are harvested and embedded in paraffin and tissue sections (4–5 μm) are stained with H&E using standard methods. Histopathological scoring can be conducted by examining 10 consecutive ×100 fields per section from at least 3 mice/group. Tubular damage is then scored by calculating the percentage of tubules that showed dilation, epithelium flattening, cast formation, loss of brush border and nuclei, and denudation of the basement membrane using a previously [28] described injury scale: 0, no damage; 1, <25%; 2, 25–50%; 3, 50–75%; and 4, >75%.

3.3 Critical notes

1. The severity of cisplatin nephrotoxicity is strain dependent and hence the dose used may have to be identified through preliminary dose–response experiments. For example, the FVB/NJ mice [28] are more sensitive to cisplatin nephrotoxicity than C57BL/6 J mice [23].

2. Female mice display more variability and are in general more sensitive to cisplatin nephrotoxicity [26].

3. 8–12-week-old male mice are ideal for cisplatin nephrotoxicity experiments.

4. It has been shown that DMSO can inactivate cisplatin [29], hence it should be avoided for preparing cisplatin or for preparation of other drugs used in combination with cisplatin.

4. Rhabdomyolysis associated acute kidney injury

4.1 Background

AKI is the most serious complication of rhabdomyolysis, representing up to 10% of all cases of AKI [4]. Although the exact mechanisms remain unknown, hypovolemia, myoglobinuria, and metabolic acidosis contribute to the pathogenesis of rhabdomyolysis-associated AKI. Importantly, myoglobinuria is likely initiating factor. Myoglobin is a 17.8-kDa iron- and oxygen-binding protein found in the skeletal muscle cells [4]. During rhabdomyolysis, myoglobin is released into the circulation, and since it is freely filtered by the glomerulus, it then enters the tubule epithelial cell through endocytosis. Traditionally, it has been believed that heme and free iron-driven hydroxyl radicals associated with myoglobin contribute to tubular damage through oxidative stress linked mechanisms [30]. Interestingly, it has been suggested recently that myoglobin can also directly promote oxidation of biomolecules, lipid peroxidation, and the generation of isoprostanes through its peroxidase-like enzyme activity [31].

Rhabdomyolysis-associated AKI can be mimicked in murine models through glycerol injection in the hind-leg muscles [27]. In this model, a single bilateral intramuscular injection (7.5 mL/kg 50% glycerol) results in development of AKI within 24 hours.

4.2 Methods

1. Prepare a 50% glycerol solution in normal saline (0.9% NaCl) followed by transferring the tube to a shaker at room temperature for 15 minutes to mix the solution. Prepare fresh solution on the day of injection.

2. Administer 50% glycerol by intramuscular injection using a 1 ml syringe with 26G needle to both the hindlimbs (once for each hindlimb). During glycerol injection, the mice are anesthetized by isoflurane administration (inhalation) before and throughout the glycerol administration procedure.

3. Immediately following glycerol injection, isoflurane inhalation is stopped, and mice are allowed to recover, followed by transfer to regular cages. Since the mice will have severe muscle injury, food, water, and painkillers (e.g., Caprofen) are provided in 1–2 oz. gel cup for up to 5 mice per cage.

4. The mice will develop AKI within 24–48 hours and at these time-points, mice can be euthanized by carbon dioxide euthanasia followed by serum and tissue collection [27]. Kidney injury can then be evaluated by biochemical analysis [blood urea nitrogen (BUN) and creatinine], histological examination [hematoxylin–eosin (H&E) staining], and analysis of renal expression of injury biomarkers such as kidney injury molecule-1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL) [32]. Blood urine nitrogen and enzymatic assay-based creatinine measurements can be performed in serum or plasma samples using commercially available kits. For histological analysis, mouse kidneys are harvested and embedded in paraffin and tissue sections (4–5 μm) are stained with H&E using standard methods. Histopathological scoring can be conducted by examining 10 consecutive ×100 fields per section from at least 3 mice/group. Tubular damage is then scored by calculating the percentage of tubules that showed dilation, epithelium flattening, cast formation, loss of brush border and nuclei, and denudation of the basement membrane using a previously described [28] injury scale: 0, no damage; 1, <25%; 2, 25–50%; 3, 50–75%; and 4, >75%.

4.3 Critical notes

1. The severity of rhabdomyolysis-associated AKI is strain dependent and hence the dose used may have to be identified through preliminary dose–response experiments. For example, the FVB/NJ mice are more sensitive than C57BL/6 J mice to rhabdomyolysis associated AKI.

2. Due to lower muscle mass, it is technically challenging to induce rhabdomyolysis associated AKI in female mice.

3. 8–12-week-old male mice are ideal for rhabdomyolysis experiments.

4. We have found no circadian related differences in severity of rhabdomyolysis associated AKI and hence glycerol can be injected at any time to initiate injury.

5. Folic acid-induced nephropathy

5.1 Background

It was reported in the late 60's that a single bolus injection of high-dose (250 mg/kg body weight) induced rampant renal hypertrophy and cell proliferation [33]. A large increase in the number of dividing cells was reported within 18 hours, with the maximum division of cells within the renal medulla occurring at 24-hours post FAN whereas the cortex reached its peak at 26 hours. This appears to be due to renal tubular epithelial damage that appear to be mediated by both the deposition of folic acid crystals and subsequent tubular obstruction as well as direct nephrotoxicity due to high-dose FAN [34]. The histological evidence of tubular injury is accompanied by reduced renal function as measured by circulating creatinine and blood urea nitrogen levels. Both increase in proportion to the dose of FAN, with the 250 mg/kg dose resulting in severe acute kidney injury with significant increase (greater than double) in circulating markers and histological evidence of acute tubular necrosis at 48 hours post injection. If the animals survive, the FAN model is also commonly used to study renal fibrosis (key finding in chronic kidney disease) that develops 7–14 days after injection [14, 35] and continues to progress weeks thereafter and can be exacerbated with repeated administration of FA [36, 37].

5.2 Methods

1. Weigh mice and calculate the amount of folic acid needed to achieve a 250 mg/kg body weight dose.

2. Dissolve the folic acid into 500 μL of 0.3 mM sodium bicarbonate for each mouse to be injected.

3. Anesthetize the animal (3% isoflurane in 100% oxygen) and sterilize the abdomen with repeated 70% ethanol/betadine wipes.

4. Invert the FA solution several times and then inject the 500 μL of the folic acid/0.3 mM FA solution into the peritoneal cavity, being careful not to puncture the gastrointestinal tract.

   a. Larger gauge needle (18-24G) will allow for easier injection as the folic acid may clog smaller needles.

   b. 500 μL of 0.3 mM sodium bicarbonate can be used as the vehicle control.

5. Allow the animals to recover from anesthesia and return to original housing once consciousness is retained.

6. Disease is evident after 24 hours, with peak evidence of kidney dysfunction occurring 36–48 hours post injection.

   a. In our experience, this is when animal mortality begins to occur.

7. 36–48 hours after injection, blood can be collected, and the animals euthanized (order depending on method of blood collected). Kidneys can be collected for quantification of tissue injury by histology or a variety of other methods. Blood can be processed to plasma or serum and used to quantify creatinine or blood urea nitrogen using commercially available assays.

5.3 Critical notes

1. This method will result in robust kidney injury in 8–12-week-old male mice of the C57BL/6 J strain (~25 grams of body weight).

2. If using females, a different strain, or older mice it is recommended to perform a pilot study to determine optimal dose.

    a. Similar protocols have been employed to induce kidney injury in rats.

    b. Older mice will develop more severe injury so a reduced dose of folic acid may be needed to assess treatment effects or avoid unintentional mortality.

3. If mice differ in body weights, it may be of value to apply the same dose (based on the animal with the smallest body weight) to avoid differences in absolute amount of folic acid administered.

    a. As an example, for more obese animals in different dietary groups

6. Sepsis and sepsis-associated AKI

6.1 Background

Sepsis is characterized by a severe inflammatory response to infection, and one of its complications is acute kidney injury. Animal models that mimic the pathophysiology of human sepsis and associated acute kidney injury are valuable because they aid in identifying molecular targets and in developing therapeutics. Too often, animal models do not properly mimic human disease. In this chapter, we describe the three commonly used approaches that have resulted in improved animal models to study sepsis.

Sepsis is a complex condition that results in a dysregulated host response to an infection and is associated with unacceptably high mortality [38]. Sepsis-associated acute kidney injury (S-AKI) is a common end organ manifestation in hospitalized and critically ill patients and is associated with high mortality and increased risk of developing chronic comorbidities [39, 40]. As individual syndromes, sepsis and AKI render the host susceptible to each other. Sepsis has a complex and unique pathophysiology, which makes S-AKI a distinct syndrome from any other phenotype of AKI. Identifying the exact onset of AKI in sepsis is nearly impossible, leading to difficulty in timely intervention for prevention of renal injury. This has limited our understanding of pathophysiologic mechanisms and precluded the development of effective therapies. The pathogenesis of S-AKI is multifactorial and involves systemic cytokine storm, hypoxia, mitochondrial dysfunction, tubular epithelial cell injury, and endothelial dysfunction [41, 42].

Local mechanisms cannot be identified and studied in humans, and hence the use of relevant animal models is paramount to eventually identify new therapeutic target to treat a syndrome still mainly managed by antibiotics and fluid resuscitations. Animal models of sepsis need to reproduce the complexity and severity of human sepsis, mimic the key hemodynamic and immunologic (proinflammatory stimulation, anti-inflammatory counter regulation, i.e., immune depression) stages as well as the modest histological findings. The sepsis inducing procedure should result in toxicity and bacteremia and should result as metabolic and physiologic

changes. Furthermore, the septic insult should manifest over sufficient length of time to allow the study of its evolution and finally the model should be reproducible and inexpensive.

Below we discuss the three commonly used animal models of sepsis and S-AKI and summarize the advantage and shortcomings of each.

7. Lipopolysaccharide injection: A model of Endotoxemia

Probably the most extensively studied animal model of sepsis is based on the intraperitoneal injection of lipopolysaccharide (LPS) [43, 44]. LPS is the major outer surface membrane component present in almost all Gram-negative bacteria and act as extremely strong stimulators of innate [45]. Although this model is simple to learn and perform, it has inherent limitations. The primary ones being that it neglects the direct host-pathogen interactions and is limited to Gram negative organism-induced sepsis. A bolus administration of LPS is essentially a model of severe inflammatory response syndrome (SIRS), rather than a true septic mimetic [46] and there is no microbial source for ongoing LPS, or pathogen associated pattern (PAMP) release. Key to the optimal success of this septic AKI model is amongst others, the use of the proper serology of LPS (E Coli. O111.B4), age of mice [43] and fluid resuscitation [47]. Compared to true human sepsis, endotoxemia results in a rapid and exponential spike in plasma inflammatory cytokines, and this SIRS like condition resolves rapidly [48–50]. This model is of scientific utility in interrogating specific biological mechanisms and pathways, such as the immune response to prototypical stimuli of specific toll-like receptor (TLR) pathways, TLR4.

7.1 Methods

1. Prepare a 1 mg/mL stock solution of LPS in sterile PBS or normal saline (0.9%NaCL). For consistent results use the same serology of LPS (*E. coli* O111. B4). If not being used immediately, aliquot and stored in - 80°C for up to 6 months. Avoid repeated freeze thaw.

2. Generally male mice between 8 and 12 weeks are used in this model, unless the effect of aging is a study parameter. Aged mice are more suspectable to LPS-induced injury. To inject mice, bring to room temperature and inject based on your experimental requirements. Dilute if required using PBS or normal saline. A low dose 1 mg/kg, intraperitoneal injection causes mild to no AKI, whereas 5 mg/kg and above results in severe SIRS and resultant AKI.

3. Depending upon the dose a spike in TNF-alpha is observed within an hour which subsequently subsides with time (at 24 hours, no to little TNF alpha is observed in the serum). Other key cytokines like IL-6 also go up by 3–4 hours. In the kidney, local changes can be observed at microscopic levels, but no signs of AKI are seen.

4. Even with the high dose of LPS, the mice look healthy and move around freely during the first 3–4 hours. However, 6 hours post treatment, mice start to assume a hunched posture, lack of movement is obvious. Clinical indicators of AKI like blood urea nitrogen (BUN) are measurable. If liquid (PBS) resuscitation is given (intradermal PBS, up to 700 μL), mice recovery and progression of AKI is mitigated.

5. 16–18 hours post high dose LPS injection, mice look morbidant and are hypothermic. Clinical measurements like glomerular filtration rate drop. Mice can be sacrificed by carbon dioxide inhalation and the serum and renal tissue can be harvested for further analysis. Kidney injury can then be evaluated by serum analysis [blood urea nitrogen (BUN) and creatinine] and examination of renal expression of injury biomarkers like kidney injury molecule-1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL). Blood urine nitrogen and enzymatic assay-based creatinine measurements can be performed in serum or plasma samples using commercially available kits.

6. Overall changes in histology are minimal. Even in severe sepsis, AKI can develop in the absence of overt histological or immune-histological changes and may be functional in nature. LPS-induced endotoxemia affects renal mitochondrial function and reduces PPARγ coactivator-1α (PGC-1α), the master regulator of mitochondrial biogenesis and metabolism and are excellent read outs for kidney pathology in this model.

7.2 Key notes

An increase in LPS-induced TNFα and TNFR1 directly damages the glomerular endothelial cell fenestrae and the glomerular endothelial surface layer [8]. We have shown that not only does LPS-induce AKI in mice, but the serum of mice injected with LPS also contains cytotoxic milieu that can directly cause renal epithelial cell death [51, 52]. Overall, these studies suggest that LPS-induced AKI can be an effect of direct activity of LPS on renal parenchyma and in parallel the SIRS like condition can result in cytokine driven renal parenchymal damage to collectively worsen outcomes. Given the dependency of this model on above mentioned variables, emphasis on the importance of testing in multiple, different animal models prior to advancing therapeutic agents into clinical trials is warranted.

8. Cecal-ligation and puncture (CLP)

Cecal ligation and puncture (CLP) is considered the gold standard model for sepsis research and one of the most frequently used procedures to induce experimental sepsis in laboratory settings [53, 54]. Unlike in LPS model, endotoxic shocks are rare in humans and sepsis origin is often localized and the CLP model mimics the nature and evolution of human sepsis [55]. After a simple procedure, CLP induces sepsis secondary to a stercoral peritonitis. This is followed by bacteremia with an early inflammatory phase, followed by an anti-inflammatory response [53]. However, significant variability in mortality from one experimental protocol to another can lead to differing interpretations of the results and S-AKI.

8.1 Methods

1. Majority of the studies in this model have been performed using male mice between 8 and 12 weeks of age. Age plays a critical role in susceptibility to injury in this model and the researchers should use appropriately aged mice depending upon their research questions.

2. Anesthetize the mice by intraperitoneal injection of a mixture of ketamine/xylazine. Isoflurane at a concentration between 3.5 to 4.5% with O_2 flow at 2 L/min can also be used. Scruff the mouse and shave the lower half of their

abdomen. Wipe done the shaved area 3 times alternating between 10% iodine and 70% ethanol. After the final ethanol wash, using either straight-edge or iris scissors, make a 1.5 cm midline cut into the skin only, approximately 0.5–1 cm away from xiphoid process.

3. Identify the abdominal wall and make another 1 cm midline cut into the peritoneum. In most instances, the cecum will be located directly under the incision. Exteriorize the cecum, align it and ligate it with a 2–0 silk suture. The cecum can be ligated at 5, 20 or 100% distal to the ileocecal valve. The ligation length is a major severity factor in CLP as the extent of the septic shock and hypotension, pro-inflammatory status, organ dysfunction, and hyperlactatemia are directly proportional to ligation length [56]. Following ligation, the cecum is punctured with a needle (generally 20–21 g) leading to leakage of fecal contents into the peritoneum. The puncture needs to be made in one pass, through and through both sides of the bowel wall. Some researchers apply pressure to release cecal contents into the peritoneal cavity. This part of the surgical manipulation is operator dependent and not well standardized across labs, resulting in differences in extent of injury and interpretation of outcomes.

4. Replace the ligated and punctured cecum into the abdomen. Close the abdominal wall with two 3–0 absorbable polyfilament interrupted sutures. Subsequently, approximate the skin using an auto-stapler. This part of the surgical manipulation is operator dependent and not well standardized across labs, resulting in differences in extent of injury and interpretation of outcomes. Inject 1 mL of saline/buprenorphine mix into the scruff of the animal's neck subcutaneously. Supportive treatment with fluids and antibiotics is too a variable and adds a new level of complexity [57]. Return the animals to their cages after they awaken from anesthesia.

5. With time, both Gram positive and negative bacterial species invade the blood stream, leading to a progressive systemic inflammatory response syndrome followed by septic shock and multiorgan injury including S-AKI [58, 59]. CLP-induced sepsis shows a cytokine profile like that observed in human sepsis [46, 60].

8.2 Key notes

CLP-induced sepsis increases lymphocyte apoptosis, which mimics immunosuppression during the late stage of human sepsis [61, 62]. In this respect, CLP-induced sepsis is completely different from LPS-induced sepsis and more closely mimics human sepsis. While we and others have reported that the CLP model can result in S-AKI [52, 63–66], AKI as measured by changes in BUN and creatinine is not always detected in this model [67, 68]. Thus, the standard CLP model amalgamates the common clinical features of human sepsis than the LPS model but misses some key features, especially acute lung injury [69] and variability in development of AKI.

9. Cecal slurry-induced peritonitis

Given the short comings and technical difficulties associated with existing models of sepsis, Gonnert *et. al* [70], developed a new model of sepsis by transferring human fecal matter into rats. This model, termed cecal slurry-induced sepsis

(CS) has since been adapted in both mice and rats, wherein the contents from the cecum of unmanipulated animals are suspended in liquid form and injected into the abdominal cavity of other animals to induce polymicrobial sepsis [71–73]. The CS model of sepsis was initially limited to those who study sepsis in neonatal mice [74], but now is also utilized to study the pathogenesis of sepsis in adult and old mice [75].

9.1 Methods

1. Generate sufficient cecal slurry by collecting cecal content from donor 16-week-old C57BL/6 mice was mixed with sterile water (0.5 mL per 100 mg cecal content). This is achieved by euthanizing the mouse by method of choice and collecting the entire cecal contents using sterile forceps and spatula. The collected cecal contents are combined, weighed, and mixed with sterile water at a ratio of 0.5 ml of water for 100 mg of cecal content. This slurry was sequentially filtered through 860-μm, 190-μm, and 70 μm mesh strainer without loss of bacteria. The filtered slurry was then mixed with an equal volume of 30% glycerol in phosphate buffered saline (PBS), resulting in a final CS stock solution in 15% glycerol.

2. Immediately after preparation of CS stock, an aliquot of CS should be serially diluted with sterile saline and plated onto multiple agar plates containing 3.7% w/v brain- heart infusion broth and 0.15% w/v agar to assess bacterial viability.

3. To induce polymicrobial sepsis using CS, mice (12–14 weeks) are used. The extent of sepsis induced is dependent on the CFU injected (from 4 to 6 X10 [4]) and age of the mice. 100 μL of CS, injected intraperitoneally is non-lethal to young and middle-aged mice but resulted in only 50% survival in aged mice [75]. The mortality increases with the volume injected. Since this model involves only a peritoneal injection, it is easy to perform and has less operator variability. Circulating bacteria levels strongly correlate with mortality in this model, suggesting an infection-mediated death.

9.2 Key notes

This model shows similar hemodynamic and physiological changes to those in human sepsis, is reproducible, and is operator-independent [76]. The development of S-AKI in this model is still understudied [77] and the induction of peritonitis by this model may not always induce kidney injury as shown recently by Shaver *et. al*[78]. However, since degree of early inflammation associated with this model is mild, it allows us to introduce other confounding like hemorrhage and investigate the finer mechanisms that can lead to S-AKI [78].

The principal reason for investigating different animal models of abdominal sepsis and its progression to S-AKI is eventually to develop therapies to treat sepsis and associated morbidities in a clinical setting. Therefore, the choice of animal model should incorporate and account for clinical caveats. Animal models of sepsis and S-AKI are essential for scientific understanding of human disease, but if they are not well characterized and understood, erroneous conclusions may be drawn, which can hinder scientific progress. A well-designed septic animal model requires a thorough understanding of the similarities and differences in the physiology of humans. To that end, the information presented in this chapter provides a systematic basis for the development of animal models for abdominal sepsis research. A perfect murine model of sepsis does not exist. While none of the current animal

models manifest and replicate the intricacies of human sepsis and S-AKI, they provide a platform for sophisticated testing that is far beyond that of cell and tissue culture.

10. Conclusions

The pathophysiology of AKI is remarkably complex, due to the involvement of multiple cells types in the endothelium, renal tubules, and the immune system. To add to this complexity, AKI occurs in the setting of other diseases such as sepsis, rhabdomyolysis, cancer, and cardiovascular disorders, wherein the underlying disease or related therapies trigger renal dysfunction through complex mechanisms that remain incompletely understood. The animal models described in the current chapter reflect and recapitulate the complexities associated with AKI and are essential tools for studying the pathophysiological mechanisms that drive acute kidney injury.

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