RESEARCH ARTICLE

A zebrafish model of infection-associated acute kidney injury

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Wen X, Cui L, Morrisroe S, Maberry D Jr, Emlet D, Watkins S, Hukriede NA, Kellum JA. A zebrafish model of infection-associated acute kidney injury. Am J Physiol Renal Physiol 315: F291–F299, 2018. First published March 14, 2018; doi:10.1152/ajprenal.00328.2017.—Sepsis-associated acute kidney injury (S-AKI) independently predicts mortality among critically ill patients. The role of innate immunity in this process is unclear, and there is an unmet need for S-AKI models to delineate the pathophysiological response. Mammals and zebrafish (Danio rerio) share a conserved nephron structure and homologous innate immune systems, making the latter suitable for S-AKI research. We introduced Edwardsiella tarda to the zebrafish. Systemic E. tarda bacteremia resulted in sustained bacterial infection and dose-dependent mortality. A systemic immune reaction was characterized by increased mRNA expressions of il1b, tnfα, cxcl8-l1, and cxcl8-1i (P < 0.0001, P < 0.001, P < 0.001, and P < 0.01, respectively). Increase of host stress response genes cdc1 and tp53 was observed at 24 h postinjection (P < 0.0001 and P < 0.05, respectively). Moderate E. tarda infection induced zebrafish mortality of over 50% in larvae and 20% in adults, accompanied by pericardial edema in larvae and renal dysfunction in both larval and adult zebrafish. Expression of AKI markers insulin-like growth factor-binding protein-7 (IGFBP7), tissue inhibitor of metalloproteinases 2 (TIMP-2), and kidney injury molecule-1 (KIM-1) was found to be activated by host-pathogen interactions, including those between pattern recognition receptors (PRRs) and invading pathogen-associated molecular patterns (PAMPs) originating from exogenous bacteria and/or danger-associated molecular patterns (DAMPs), endogenous “alarmins” released from injured tissue. Although established models in higher vertebrates have successfully recapitulated the major features of human S-AKI (9), there is a continuous and pressing need for additional tools to better understand the signaling networks involved. In addition, the interaction between kidney damage and dysfunction and their association with innate immune activity remain unclear.

The zebrafish (Danio rerio) has an immune system that is similar to that of humans (43). However, larvae possess only innate immunity, whereas the adaptive immune system does not mature until the juvenile stage, at 4 wk postfertilization (27). Given this developmental feature, zebrafish larvae have been used as a model to achieve several breakthroughs in the understanding of host-pathogen interactions that would have been difficult to realize with traditional models (46). In addition, zebrafish kidney develops and functions in almost the same way as that of humans: their key pronephros genes have been identified as homologous to those of humans (14). The larval pronephros starts functioning as early as 48 h postfertilization (hpf; 10). Moreover, nephrotoxic AKI and laser ablation-induced AKI have been successfully developed using zebrafish larvae (17).

We sought to establish a zebrafish model of S-AKI. By challenging zebrafish with a fish-specific pathogen, we tested whether zebrafish develop S-AKI and whether PAMPs invasion and activated innate immunity alone could cause AKI in zebrafish larvae. Successful development of this model will enrich the S-AKI study toolkit, facilitating better understanding of the mechanisms of S-AKI.

MATERIALS AND METHODS

Design. The study was designed to induce a systemic inflammation (sepsis) with controllable mortality, to identify signs of AKI in both larval and adult zebrafish. All studies of larvae were performed at the age of 78–82 hpf, and adult zebrafish studies were performed at the age of 1 yr. Male and female animals were randomly assigned to study groups.

Animals. Zebrafish [Pitt AB and the transgenic lines Tg(Pt::eGFP) and Tg(cdh17::eGFP)] were raised at 28.5°C under a 14:10-h light-dark cycle in regular tank water at the University of Pittsburgh zebrafish facility (6). Procedures were conducted per the National...
Institutes of Health Guide for the Care and Use of Laboratory Animals. Experimental adult zebrafish and larvae were incubated in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, and 0.33 mM MgSO₄, buffered to pH 7.2 with 10 mM HEPES) and maintained according to The Zebrafish Book (50). Healthy adult zebrafish (Pitt AB, 1 yr old) were used for adult experiments. Larvae were obtained from spawning eggs, incubated in E3 medium-0.01 mg/l methylene blue (Sigma-Aldrich) or 0.005% 1-phenyl-2-thiourea (Sigma-Aldrich) for 78–82 h, and then subject to experiments. To deactivate the translational activity of the target gene, morpholino antisense oligonucleotides (0.50 mM with 0.05% phenol red; Gene Tools) were injected into zebrafish embryos. Morpholino oligonucleotide sequences complementary to igfbp7 and timp2 translation-blocking targets were 5'-GGCGAGGCGAAGACACACACAGCT-3' and 5'-ACACGCTCTGACGCTCTTCTATTTT-3', respectively. Standard morpholino control oligonucleotides were injected as controls. Tricaine (MS-222; Sigma-Aldrich) in E3 medium (buffered to pH 7 with 0.64 mg/ml). Bacterial infection. Edwardsiella tarda (Ewing and McWhorter O1483:H1; ATCC 15947; American Type Culture Collection) were microinjected into the larval bloodstream through the duct of Cuvier. After culturing in lysogeny broth medium (Sigma-Aldrich) overnight at 37°C with shaking, the bacteria were centrifuged and resuspended in PBS-0.2% phenol red (Sigma-Aldrich) to the desired concentrations. To induce different severities of infection, an inoculum of E. tarda, 20 μl for adult fish (administered intraperitoneally) and 1 nl for larvae (administered intravenously), at incremental concentrations was injected into zebrafish, and zebrafish were observed for 7 days. Based on mortality rate, inocula of 6×10⁴ colony-forming units (CFU) E. tarda, which induced 20% mortality in adult zebrafish, and 300 CFU E. tarda, which induced over 50% mortality in larvae, were selected as moderate infection dosages for the following studies. All infection pathological changes were obtained from moderate infection samples unless otherwise stated. The same volume of vehicle (0.2% phenol red-PBS) was also injected into another group of animals serving as noninfected controls. To determine the bacterial burden, the number of CFUs was quantified in infected zebrafish larvae. Specifically, groups of five larvae were triturated by repeated pipetting in 100 μl of PBS each and repeating 1% Triton X-100 (Sigma-Aldrich). Serial dilutions of this suspension were then cultured on streptomycin-selective agar plates for CFU assessment.

RNA extraction and quantitative PCR. After being injected with E. tarda, larvae were euthanized and snap-frozen at 24 h post-bacterial injection. To obtain sufficient amounts of RNA, samples of 50 larvae were pooled and subjected to TRIzol (Invitrogen) and chloroform extraction. Samples was then precipitated with isopropanol, washed in 75% ethanol, and suspended in diethyl pyrocarbonate-treated water. After purification with RNeasy Mini Kit (Qiagen), RNA concentration was measured using the NanoDrop system (Thermo Fisher Scientific). Sample DNA was degraded using DNase I (Thermo Fisher Scientific), and cDNA was subsequently prepared with a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative PCR was performed on a Stratagene MX3000P instrument (Agilent Technologies) using SYBR Select Master Mix (Thermo Fisher Scientific). The primers were used on the basis of previous publications (28, 36, 52) or designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3_2; Table 1). Fold changes in the expression of each target gene normalized to that of gadd45a were determined as follows: fold change = 2^(-ΔΔCt) where ΔCt in threshold cycle (ΔCt) = Ct_target − Ct_gadd45a and ΔΔCt = ΔCt_injected − ΔCt_control. Each sample was processed in duplicate for quantification of expression.

RNA imaging. Live imaging was used to assess kidney excretion function in larvae (17, 33). Specifically, anesthetized larvae were injected into the duct of Cuvier with 40-kDa FITC-dextran (Sigma-Aldrich) at a concentration of 10 mg/ml 2 days after injection of bacteria. To observe changes over time, larvae were anesthetized, transferred to a depression slide, oriente, immobilized in 2% methylcellulose (Sigma-Aldrich) in E3 medium, and imaged under a Z1X10 stereomicroscope (Olympus). Bright-field images were obtained 3, 24, 48, and 72 h after injection of bacteria for morphological analysis of edema-like formations. Intensities of in vivo fluorescence in larval pericardia were measured 3 and 24 h after injection of FITC-dextran to evaluate kidney function. Larvae were anesthetized for no longer than 15 min to ensure their vitality and were returned to fresh E3 medium to recover. Fluorescence intensities were quantified using ImageJ (https://imagej.nih.gov/ij/; National Institutes of Health) by measuring average grayscale values in a fixed region of interest of equal size across images and processed with Photoshop (Adobe Systems). Cardiac rates were also recorded by visual inspection. The baseline was arbitrarily set at 150 beats/min, and the heart rate index was acquired from the ratio of actual rate to baseline rate. Normalized renal clearance index was expressed as the total excreted fluorescence normalized by the heart rate index, as follows: pro nephric filtration capacity = (FITC intensityy3h – FITC intensityy2h)/heart rate index.

**Renal tubular endocytosis.** Tubular endocytosis was evaluated for tubular epithelial cell injury and dysfunction. Similar to mammalian proximal tubular epithelial cells in the kidney, zebrafish nephrons express an endocytic receptor, megalin/LDL receptor-related protein-2 (LRP2), which plays a crucial role in renal endocytic machinery. Anzenberger et al. reported that 70-kDa dextran was taken up through megalin/LRP2 endocytic receptor in the zebrafish nephron after injection (1). The reabsorbed dextran penetrates the intercellular spaces and tight junctions of cells from the peritubular capillaries; the latter are permeable to all dextran fractions (4). Briefly, 70-kDa tetramethylrhodamine isothiocyanate (TRITC)-dextran (Sigma-Aldrich; 20-μl volume at a concentration of 25 mg/ml in 0.05% phenol red-PBS) was injected into anesthetized adult zebrafish at 1 day postinfection. Uptake of dextran by the nephron tubule segment was examined 18–24 h post-dextran injection. Zebrafish were euthanized, and the abdomen was immediately opened by making a ventral incision from the head to the base of the caudal fin. After removing the internal organs,
the nephron was exposed, detached from the dorsal wall, and washed three times in PBS. The zebrafish nephron was then flat mounted in PBS on slides covered by a glass coverslip with modeling clay on each corner and subject to immediate examination under a 2X10 stereomicroscope (Olympus).

**Immunofluorescence staining on whole mount larvae and cryosections.** For whole mount immunofluorescence staining, euthanized larvae were dissected in ice-cold PBS, removing as much unwanted abdominal tissue as possible. Samples were then fixed in an ice-cold solution of 4% (wt/vol) paraformaldehyde and 0.1% DMSO in PBS overnight at 4°C, before being treated with a series of sucrose solutions (10, 20, and 30% in PBS). After incubating overnight in 30% sucrose in PBS for permeabilization, samples were exposed to 5 μg/ml proteinase K in PBS containing 1% Triton X-100 (PBST) for 3 min for antigen retrieval and blocked with 10% FCS and 0.2% sodium azide in PBST for 2 h at room temperature. Primary and secondary antibodies in 2% FCS and 0.02% sodium azide in PBST were incubated sequentially with samples by gentle rotation at 4°C for 3 min for antigen retrieval and blocked with 10% FCS and 0.2% sodium azide in PBST for 2 h at room temperature. Primary and secondary antibodies in 2% FCS and 0.02% sodium azide in PBST were incubated sequentially with samples by gentle rotation at 4°C for 2–4 days. Typically, samples were washed three to five times with 0.2% sodium azide in PBST between each step to thoroughly remove residual material. Stained samples were equilibrated in 100% glycerol for 48 h until examined.

For immunofluorescence staining on larval cryosections, larvae were euthanized, fixed with aceton at −20°C for 15 min, embedded with tissue-freezing medium (Tedpella) in cryomolds (Tedpella), orientated to the proper position, frozen on dry ice for ~30 min, and stored at −80°C until sectioned. Samples were then sectioned (10 μm thick) through the entire larva and then air-dried completely for 30 min, rehydrated with 0.1% Tween 20-PBS (PTw) three times for 5 min, and then blocked with 10% sheep serum (Sigma-Aldrich) in PTw for 1 h at room temperature. Section samples were incubated with primary antibodies at 4°C overnight and then secondary antibody at room temperature for 1.5 h. PTw washes were performed three times between each step. The slides were overlaid with mounting medium with DAPI (Vector Laboratories), covered with coverslips, and stored at −20°C until observation under a Fluoview FV1000 confocal microscope (Olympus). Images were processed using ImageJ and Photoshop.

Primary antibodies were used at a dilution of 1:100: rat anti-mouse kidney injury molecule-1 (KIM-1; Novus Biologicals), rabbit anti-human insulin-like growth factor-binding protein-7 (IGFBP7; Santa Cruz Biotechnology), and mouse anti-human tissue inhibitor of metalloproteinases 2 (TIMP-2; Abcam). Fluor-conjugated anti-rat, anti-rabbit, and anti-mouse secondary antibodies were used at a dilution of 1:1,000 (Jackson ImmunoResearch Laboratories).

**Pathway analysis.** The associated functions were generated from the seven significant genes documented in this paper. Downstream effects (26) were predicted through the use of IPA (QIAGEN, https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis).

**Statistical analysis.** Graphing and statistical tests to compare groups were performed using GraphPad Prism 7 (GraphPad Software). Kaplan-Meier curves are presented as percentages of surviving animals over 7 days, and the significance of difference was assessed by log-rank test. Numeric results are presented as means ± SE, and group differences were tested using two-tailed t-tests. P < 0.05 was considered an indication of significant difference.

**Ethics approval.** Procedures involving adult and larval zebrafish were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

**RESULTS**

To establish an infection model in zebrafish, we injected *E. tarda* into 1-yr-old adult zebrafish or larvae at 78–82 hpf at the duct of Cuvier (Fig. 1). Bacterial load increased significantly in infected larvae [starting from 300 CFU and increasing to 600 and 7,333 CFU] from 24 to 72 h post-bacterial injection (Fig. 1B). Microinjection with 300 CFU or more caused 60–90% of larvae to die in a dose-dependent manner (P < 0.0001), whereas infection with 100 CFU did not result in significantly increased mortality compared with the PBS-injected controls. Adult zebrafish injected with 6 × 10⁷ CFU *E. tarda* exhibited significantly increased mortality compared with controls (Fig. 1C). The appearance of sepsis was characterized by systemic increased immune mediators. Figure 1D shows mRNA fold increase for inflammatory cytokines. Expression of chemokine (C-X-C motif) ligand 8 (cxcl8-1l), interleukin-1β (il1b), tumor necrosis factor-α (tnfa), and transforming growth factor-β1a (tgfb1a) in *E. tarda* septic larvae was significantly increased compared with control animals (P < 0.01, P < 0.0001, P < 0.001, and P < 0.001, respectively). Transcriptional upregulation of stress response genes cyclin D1 (ccnd1) and tumor protein p53 (tp53) in septic larvae was significantly higher (P < 0.001 and P < 0.05, respectively) compared with control animals at 24 h post-bacterial injection (Fig. 1D).

Infected larvae displayed visible edema compared with controls, which suggests fluid retention due to kidney dysfunction (Fig. 2A). To confirm this kidney dysfunction in septic zebrafish, we administered fluorescent dextran to the animals and compared small-molecular-mass dextran intensity (40-kDa FITC-dextran) in circulation for glomerular filtration rate (GFR; Fig. 2B) and middle-molecular-mass dextran intensity (70-kDa TRITC-dextran) in nephron tubules for tubular epithelial cell injury (Fig. 2D). Significantly higher circulating fluorescence intensity was measured in septic larval pericardium at 24 h post-dextran injection compared with control animals (P < 0.05), suggesting reduced renal clearance capacity (P < 0.001; Fig. 2C). Decreased glomerular perfusion pressure, interstitial edema, and tubular obstruction are believed to be among the main pathophysiological mechanisms for decreased GFR in both human and rodent models of S-AKI (2), and any pathological changes in septic animal nephrons could cause a GFR impairment. To test the functional integrity of tubular epithelial cells, TRITC-dextran (70 kDa) was injected into adult zebrafish. Adult zebrafish were used to confirm translatability of S-AKI from larvae to adults and to visualize cellular uptake in a multinephron system (28). TRITC-dextran (70 kDa) is filtered slowly through the glomerulus for decreased GFR in both human and rodent models of S-AKI (70-kDa TRITC-dextran) was in nephron tubules for tubular epithelial cell injury (Fig. 2D). Decreased glomerular filtration rate compared small-molecular-mass dextran intensity (40-kDa FITC-dextran) in circulation for glomerular filtration rate (GFR; Fig. 2B) and middle-molecular-mass dextran intensity (70-kDa TRITC-dextran) in nephron tubules for tubular epithelial cell injury (Fig. 2D). Significantly higher circulating fluorescence intensity was measured in septic larval pericardium at 24 h post-dextran injection compared with control animals (P < 0.05), suggesting reduced renal clearance capacity (P < 0.001; Fig. 2C). Decreased glomerular perfusion pressure, interstitial edema, and tubular obstruction are believed to be among the main pathophysiological mechanisms for decreased GFR in both human and rodent models of S-AKI (2), and any pathological changes in septic animal nephrons could cause a GFR impairment. To test the functional integrity of tubular epithelial cells, TRITC-dextran (70 kDa) was injected into adult zebrafish. Adult zebrafish were used to confirm translatability of S-AKI from larvae to adults and to visualize cellular uptake in a multinephron system (28). TRITC-dextran (70 kDa) is filtered slowly through the glomerulus for decreased GFR in both human and rodent models of S-AKI (70-kDa TRITC-dextran) was in nephron tubules for tubular epithelial cell injury (Fig. 2D). Collectively, these data point to nephron damage in the septic animals. Although extrarenal factors (i.e., hemodynamic changes) may also affect GFR results, such changes would still be indicative of septic AKI.
larvae. Fold changes of *kim1*, *timp2*, and *igfbp7* mRNA were significantly increased (compared with control animals: \(P < 0.05\), \(P < 0.05\), and \(P < 0.01\), respectively) in septic animals, in contrast to no significant changes in the chronic fibrosis-associated gene collagen, type I, \(\alpha 1\) (*colla1a*) levels (compared with control animals: \(P > 0.05\); Fig. 3A). To identify the spatial expression of these markers, we colocalized staining of the above-mentioned proteins with the transgenic zebrafish lines *Tg*(PT::*eGFP*) (7) or *Tg*(*cdh17::*eGFP*) (37), which fluorescently label cells in the proximal pronephric tubule or the whole length of the pronephric tubule, respectively (Fig. 3B). Our results showed increased expression of *TIMP-2* and *IGFBP7* in the pronephros at 48 h post-bacterial injection, with *TIMP-2* predominantly found in the distal tubule and *IGFBP7* in the pronephros at 48 h post-bacterial injection, with *IGFBP7* in the proximal tubule. Increased *KIM-1* expression was detected in the whole length of the pronephros tubule in the septic larvae (Fig. 3B). We thus confirmed the expression of tubule injury markers in septic larvae at both protein and mRNA levels, which was consistent with the decline in pronephric filtration function. To dissect the possible associations among injury markers, *igfbp7* and *timp2* translations were inactivated by corresponding morpholino injections. Immunofluorescence staining showed that *KIM-1* protein expression in infected larvae remained present in *TIMP-2*\(^{-/}\) animals whereas absent in *IGFBP7*\(^{-/}\) animals (Fig. 3C). These data suggest that all three markers, *IGFBP7*, *TIMP-2*, and *KIM-1*, were upregulated in response to infection and had increased expression at the protein level in the septic kidney tubule; expression of *KIM-1* is affected by *IGFBP7* but not by *TIMP-2*.

To better understand possible functions and generate hypotheses for increased expression of markers during AKI, we performed pathway analyses. Interaction between the pathogen and Toll-like receptors (TLRs), which are classic PRRs, upregulated *il1b*, *tp53*, and *ccnd1*; associated with events of DNA damage, cell migration, movement of phagocytes, and inhibited cell differentiation. Notably, *TIMP-2* expression could increased as a consequence of TLR pathway activation. The analysis also suggested that *KIM-1* [hepatitis A virus cellular receptor 1 (HAVCR1)] may promote cell migration and phagocyte movement, whereas *TIMP-2* and *IGFBP7* appear to inhibit these processes. *KIM-1* and *IGFBP7*, but not *TIMP-2*, inhibited cell differentiation (individual results not shown). The overall consequence of activating *KIM-1*, *IGFBP7*, and *TIMP-2* in addition to TLR activation likely was promotion of cell migration and phagocyte movement and inhibition of cell differentiation (Fig. 3D).

In summary, a systemic zebrafish infection was induced by injection of the bacteria *E. tarda* in both larval and adult
animals. Similar to adult zebrafish nephron injuries, septic zebrafish larvae with only innate immunity resulted in characteristic features of AKI. Pathway analysis suggested that the effects of KIM-1 are in opposition to those of IGFBP7 and TIMP-2 on cell migration and phagocyte movement whereas both IGFBP7 and KIM-1 have inhibitory effects on cell differentiation after injury.

DISCUSSION

We established a zebrafish model of S-AKI by administration of the fish-specific pathogen, E. tarda bacteria. This model recapitulated several key features of human S-AKI, including cytokine upregulation, host transcriptional response to injury, decreased kidney function, and increased expression of tubular injury biomarkers. Given that sepsis is a major cause of AKI, a zebrafish model of S-AKI is urgently needed and will hopefully serve as a highly useful complement to higher-vertebrate models.

Zebrafish, with a functional pronephros by 2 days postfertilization (10), appear to be an ideal model system for S-AKI research. Their nephron segment pattern and cellular composition are similar to those of mammalian nephrons (14). In addition, transgenic animals are being applied for precise determination of the location, function, and expression profiles of particular renal cells (46). Tg(cdh17::eGFP) and Tg(PT::eGFP) are transgenic lines that express enhanced green fluorescent protein (eGFP) driven by the tubule-specific gene promoters. The zebrafish homolog of mammalian kidney-specific cadherin, cdh17, has been reported to be expressed in the epithelium and ducts of the entire tubule during larval development and adulthood (19, 37). Tg(PT::eGFP) zebras express eGFP in only the proximal tubules (5, 7). Several kidney injury models have been established using zebrafish in recent years, successfully recapitulating the features of mammalian AKI, including typical histological changes, reduced renal function, and pericardial edema (6, 17, 23). In the present...
study, we documented infection-associated kidney dysfunction in both adult and larval zebrafish (Fig. 2). Typical function changes included nephron excretion impairment and tubular endocytosis dysfunction (Fig. 2), accompanied by traditional injury marker expressions in the pronephric tubule (Fig. 3, A and B). The anatomic localization of IGFBP7 (proximal tubule) and TIMP-2 (distal tubule) matches that recently described in humans (11).

To induce zebrafish infection, multiple pathogens have been tested. Zebrafish did not respond well to typical pathogens such as *Escherichia coli* or lipopolysaccharide, which are commonly used to induce sepsis in mammalian models (data not shown). These findings are consistent with the previous reports stating that *E. coli* can be completely cleared by the zebrafish immune system (18). In addition, lipopolysaccharide was unable to be recognized by zebrafish paralogs of TLR4, TLR4a,
and TLR4b because of structural differences in their extracellular domains (38). *E. tarda* bacteria are gram-negative aquatic pathogens that can be used to induce reproducible systemic infection in zebrafish (35, 49). *E. tarda* invades zebrafish tissues by inhibiting host lysozyme activity, resisting the complement system, and surviving inside macrophages (29, 42). *E. tarda* caused systemic infection and dose-dependent mortality in our zebrafish study (Fig. 1, B and C). The affected animals exhibited a milder manifestation than that seen in *Edwardsiella* septicemia (35, 42). Our data showed that the induced infection manifested sustained bacterial load and a globally increased proinflammatory transcriptional response involving *il1b, cxcl8-lii, tnfa*, and *tgfb1a*. The *il1b* expression may imply the activation of the inflammasome formation pathway after PRR activation by PAMPs and DAMPs; increased transcription of *il1b* is also thought to be linked to macrophage activation, which is associated with sepsis-induced tissue injury (15). To further confirm the systemic infection, host response gene expression was also tested. Transcriptional changes of the cell cycle regulatory genes *tp53* and *ccndl* were examined postinfection. Activation of *tp53* leads to cell cycle arrest; *ccndl* promotes differentiated cells reentering the cell cycle and starts repair processes (45).

This model, for the first time to our knowledge, confirmed the association between the activated innate immune response and kidney injury. Increasing evidence shows that innate immunity might play crucial roles in determining sepsis outcomes (12, 24, 25, 39). In sepsis, innate immune responses are thought to be primarily governed by factors affecting the recognition of PAMPs by PRRs, including the virulence of the invading pathogen, initial pathogen load, and host genetics (12). Hyperinflammatory or persistent inflammatory states could be caused by overreactions following recognition of PAMPs and/or DAMPs by PRRs, potentially leading to multitorgan (44) and kidney injuries (13, 21, 22). However, owing to the intricacy of the immune interactions that occur in sepsis, the effect of innate immunity on kidney injury is often difficult to ascertain in traditional mammalian models (3, 9, 22). The zebrafish innate immune system is homologous to that of mammals and widely used in understanding the role of immunity in human infectious diseases (32, 43, 47, 48). Larvae present innate immunity from 1 day postfertilization, whereas adaptive immunity is not active until 4 wk postfertilization (27, 43), providing a unique window to delineate the role that innate immunity plays in the process of infection and associated organ injury. By analyzing septic larvae, our results indicate that activation of the innate immune response alone could cause S-AKI in this model system. S-AKI is thought to contribute to sepsis mortality not only because of the nephron dysfunction itself but also because of the increased number of failure organs mediated by AKI cross talk with other organs through soluble inflammatory mediators (8, 40, 51).

In summary, we established a zebrafish model of S-AKI and confirmed the crucial role that innate immune activation plays in this condition. Despite significant differences in the physiology of zebrafish compared with mammals (20, 48), we consider the model a significant complement to vertebrate models of S-AKI not only because of similar pathologic features but also because of the unique advantages of the zebrafish. The larval model inherits important benefits from the zebrafish’s fecundity, rapid development, optical transparency, and availability of a rich variety of tools for genetic manipu-

lution. It is also less time consuming and requires less effort compared with classic mammalian models. Future S-AKI studies may seek to include other components of the innate immunity (the complement cascade, dendritic cells, T cells and microphages, morphology/phenotype analyses of the cilia or ciliated cells), podocytes, endothelial cells, slit diaphragm, and mesangial cells, which are all thought to be key elements in the initiation and progression of S-AKI (31, 53). The model could also be directed toward immune effector cell-tubule cell interactions, mechanisms underlying host cell injury and recovery, large-scale drug screens, and other research that would be difficult and/or prohibitively expensive to execute in traditional mammalian models.

**DISCLAIMERS**

The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

**AUTHOR CONTRIBUTIONS**

X.W., N.A.H., and J.A.K. conceived and designed research; X.W., L.C., S.M., D.M., and D.E. performed experiments; X.W., L.C., and S.W. analyzed data; X.W., N.A.H., and J.A.K. interpreted results of experiments; X.W., L.C., and D.M. prepared figures; X.W. drafted manuscript; L.C., N.A.H., and J.A.K. edited and revised manuscript; X.W., L.C., S.M., D.E., S.W., N.A.H., and J.A.K. approved final version of manuscript.

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