Renal microvascular endothelial cell responses in sepsis-induced acute kidney injury

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Published in:
Nature Reviews Nephrology

DOI:
10.1038/s41581-021-00489-1

Publication date:
2022

Renal microvascular endothelial cell responses in sepsis-induced acute kidney injury

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Abstract | Microvascular endothelial cells in the kidney have been a neglected cell type in sepsis-induced acute kidney injury (sepsis-AKI) research; yet, they offer tremendous potential as pharmacological targets. As endothelial cells in distinct cortical microvascular segments are highly heterogeneous, this Review focuses on endothelial cells in their anatomical niche. In animal models of sepsis-AKI, reduced glomerular blood flow has been attributed to inhibition of endothelial nitric oxide synthase activation in arterioles and glomeruli, whereas decreased cortex peritubular capillary perfusion is associated with epithelial redox stress. Elevated systemic levels of vascular endothelial growth factor, reduced levels of circulating sphingosine 1-phosphate and loss of components of the glycocalyx from glomerular endothelial cells lead to increased microvascular permeability. Although coagulation disbalance occurs in all microvascular segments, the molecules involved differ between segments. Induction of the expression of adhesion molecules and leukocyte recruitment also occurs in a heterogeneous manner. Evidence of similar endothelial cell responses has been found in kidney and blood samples from patients with sepsis. Comprehensive studies are needed to investigate the relationships between segment-specific changes in the microvasculature and kidney function loss in sepsis-AKI. The application of omics technologies to kidney tissues from animals and patients will be key in identifying these relationships and in developing novel therapeutics for sepsis.

Immune paralysis
A state in which immune cells show reduced responses to stimuli. Immune paralysis can occur in response to an overload of antigen and results in susceptibility to secondary infection. The cause of immune paralysis in sepsis is not known.

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https://doi.org/10.1038/s41581-021-00489-1

The development of acute kidney injury (AKI) in the course of sepsis is a well-known but poorly understood complication. Both AKI and sepsis are defined using clinical symptoms rather than characterized by pathognomonic signs or symptoms[1]. AKI is defined by loss of kidney function, an increase in serum creatinine (SCr) levels and/or loss of urine production[2], whereas sepsis is defined as a life-threatening loss of organ function caused by a dysregulated host response to infection[3]. Both definitions are sensitive as they include most patients with these conditions; however, not all patients who are identified using these definitions actually have AKI or sepsis. The definition of sepsis-induced acute kidney injury (sepsis-AKI) therefore captures a heterogeneous group of diseases. The incidence of AKI among patients admitted to hospital varies from 2% to 50% depending on the definition of AKI used, local hospital circumstances and health care system, and the patient population[2]. The annual incidence of sepsis is estimated to be 48.9 million worldwide, with a mortality of 11.0 million in 2017 (REF.[4]). In high-income countries, sepsis-AKI often occurs in patients with serious comorbidities including cancer, chronic kidney disease, immune suppression and vascular disease[5].

The clinical presentation of sepsis-AKI entails an initial phase of renal hyperfiltration and proteinuria followed by oliguria and decreased glomerular filtration rate (GFR). The pathophysiological mechanisms that underlie sepsis-AKI are not well understood[6]. Sepsis is frequently accompanied by hypotension and insufficient oxygen supply to the organs, a condition known as shock. As renal tubuli have a marginal oxygen supply and a high oxygen consumption under normal conditions, they are prone to oxygen deficit and consequent tubular necrosis; acute tubular necrosis has long been synonymous with sepsis-AKI[7]. Furthermore, an overwhelming immune response and immune paralysis have been suggested to underlie kidney injury and function loss in sepsis-AKI[8]. Various interventions directed at these mechanisms have been tested in randomized controlled trials, but to date none of these approaches has been shown to improve patient survival. This failure has raised doubts about the validity of these processes as key targets for sepsis-AKI treatment[9]. Moreover,

Key points

- Microvascular endothelial cells in arterioles, glomeruli, peritubular capillaries and postcapillary venules in the kidney cortex have an intrinsic molecular and phenotypic heterogeneity and respond to sepsis-induced acute kidney injury (sepsis-AKI) conditions in a segment-specific manner.
- Clinical data suggest endothelial engagement in sepsis-AKI, although the contribution of the renal microvasculature is not yet clear; changes in the levels of soluble markers in blood and urine represent endothelial responses that can originate from any organ involved in sepsis pathophysiology.
- Not all molecules that control microvascular permeability and leukocyte recruitment in organs other than the kidney have a similar role in sepsis-AKI; this heterogeneity prevents extrapolation of mechanisms reported in other organs to the kidney.
- As endothelial cells rapidly lose their behaviour in culture, molecular mechanisms or cell responses to sepsis-AKI conditions and pharmacological interventions that are identified in vitro must be validated in vivo.
- Analysis of post-mortem kidney samples from patients with sepsis-AKI as well as serial plasma and urine samples and clinical data are required to effectively translate findings from animal models to the human disease.
- Molecularly mapping endothelial responses in time in various organs will provide a rational basis for the selection of molecules that can potentially serve as (soluble) biomarkers of endothelial responses in sepsis-AKI following validation in clinical samples.

histopathological studies of kidney tissue from patients who died as a result of sepsis have not shown overwhelming necrosis or inflammation[10].

Elevated plasma levels of soluble adhesion molecules, angiopoietin 2 (ANG2) and soluble receptor tyrosine kinase TIE2 (also known as angiopoietin 1 receptor or tyrosine-protein kinase receptor TEK) in patients suggest that endothelial cells in the kidney microvasculature have a role in sepsis pathophysiology[11–13]. These cells engage in the transport of molecules — the core function of the kidney — and have a crucial role in the control of organ perfusion, anticoagulant–procoagulant balance, microvascular permeability and leukocyte recruitment, which are intrinsic components of vascular integrity. All of these processes are affected by infection and loss of kidney function[14,15].

In this Review, we discuss the role of endothelial cells in the kidney microvasculature in the development of sepsis-AKI. We begin with a brief introduction to microvascular functions in the kidney and the molecular systems that are used by endothelial cells to control vascular integrity and the responses to systemic challenges that occur in sepsis. We then discuss endothelial heterogeneity in the renal microvasculature and endothelial cell responses in the kidney in experimental models of sepsis-AKI and in human sepsis. Finally, we provide an outlook regarding experimental designs to advance endothelial biomedicine research and potentially identify novel therapeutic targets.

Vascular integrity
The homeostatic condition of blood vessels in which endothelial cells in their natural environment regulate tissue perfusion, anticoagulant–procoagulant balance, microvascular permeability and leukocyte recruitment for immune surveillance purposes.

Perm-selective barrier
The barrier formed by the glomerular endothelial cells that restricts the passage of proteins but enables passage of small molecules and water.

Microvascular functions in the kidney

The renal vasculature consists of a unique series of segments that execute dedicated tasks required for normal kidney function with anatomically separate microvasculatures in the cortex and medulla. The main function of the renal artery and its microvascular branches is to deliver blood to the kidney. Here, the renin–angiotensin system has an important role in controlling renal vascular resistance, which directly affects glomerular filtration pressure and subsequently blood flow. The microvasculature in the glomerulus filters the blood to eliminate soluble molecular waste products by formation of a perm-selective barrier, whereas peritubular microvessels engage in on-demand secretion of molecules from the blood into the urine and reabsorption of molecules from the pre-urine back into the blood[16,17]. The different functions of cortical peritubular capillaries are defined by their anatomical association with convoluted S1 and S2 segments of the proximal tubules and the straight S3 proximal tubule segment that traverses from cortex to medulla[18]. As subsegmental differences in the function and molecular make-up of endothelial cells in these peritubular microvessels are understudied, they are discussed as a single group in this Review. After traversing the kidney, cortical postcapillary venules deliver the blood back to the bigger veins[16,17].

The renal microvasculature shares a number of general functions with those of other organs, including the delivery of sufficient oxygen and nutrients to cells in the tissue, maintenance of endothelial monolayer integrity, preservation of anti-coagulant versus pro-coagulant balance and facilitation of leukocyte recruitment. To control blood flow, arteriolar endothelial cells produce nitric oxide (NO), which leads to calcium flux changes and consequent microvascular relaxation in neighbouring smooth muscle cells (SMCs)[19]. The molecular systems that endothelial cells use to maintain vascular integrity include the vascular endothelial growth factor (VEGF)–VEGF receptor (VEGFR) complex, TIE2 and its ligands ANG1 and ANG2, and the adhesion molecules platelet endothelial cell adhesion molecule 1 (PECAM1; also known as CD31) and vascular endothelial (VE)-cadherin, which, together with junctional adhesion molecules, are organized into tight junctions and adherens junctions[20–22].

Antithrombotic activity is regulated by endothelial thrombomodulin (TM), which binds to thrombin and thereby prevents its interaction with platelets and coagulation factors. TM also mediates activation of protein C, which in turn degrades coagulation factors and creates an anti-inflammatory and barrier-stabilizing status of endothelial cells via endothelial protein C receptor (EPCR) and protease-activated receptor 1 (PAR1)[23,24].

Blood vessel homeostasis and renal microvascular permeability control are further regulated by the endothelial surface layer[25], which consists of membrane-bound glycocalyx composed of proteoglycans with loosely incorporated secreted proteoglycans, glycosaminoglycans, polysaccharides and plasma proteins[26]. Fenestrated endothelial cells in the glomeruli are covered by glycocalyx in the capillary lumen that extends into the pores and also covers the podocytes[27]. Although it might seem reasonable to assume that the molecular systems that control these general endothelial functions are shared between endothelial cells in different microvascular segments, studies describing the location of proteins involved in these functions show that in the kidney this assumption does not hold true.

Endothelial heterogeneity in the kidney

All renal microvascular segments are covered at the luminal side by endothelial cells, which are surrounded by support cells at the abluminal side. SMCs support

Dwell time
The time that leukocytes spend in a particular vascular segment in a tissue in vivo. Dwell time is assessed using intravital microscopy technology.

Epigenetic mechanisms
Mechanisms of control of cellular phenotype that do not involve the sequence of DNA nucleotides. Epigenetic mechanisms include chemical modifications such as methylation of DNA and histones that affect the accessibility of the DNA for transcription.

endothelial cells in arterioles and postcapillary venules, podocytes and mesangial cells support the intricate structure of the glomerulus, and pericytes surround and support peritubular capillaries[16,28] (FIG. 1). The diversity in types of support cells along the microvasculature in the kidney provides an important basis for the microenvironmental control of endothelial cell function.

Different shear stress patterns created by local blood flow provide additional cues that influence endothelial cell behaviour[29]. As the diameter of the vessels dramatically decreases from ~30 µm in the bigger branches of the afferent arterioles to ~4–6 µm in the glomerular, efferent arteriolar, and peritubular capillary beds, endothelial cells must physically interact with cells in the blood, as evidenced by the substantial dwell time of neutrophils and monocytes in glomerular capillaries in healthy kidney[30].

The heterogeneity of endothelial cell behaviour in different microvascular segments is thought to be partly determined by the microenvironment in which the cells reside and partly by epigenetic mechanisms[31–33]. For example, differences in blood velocity and blood flow-driven

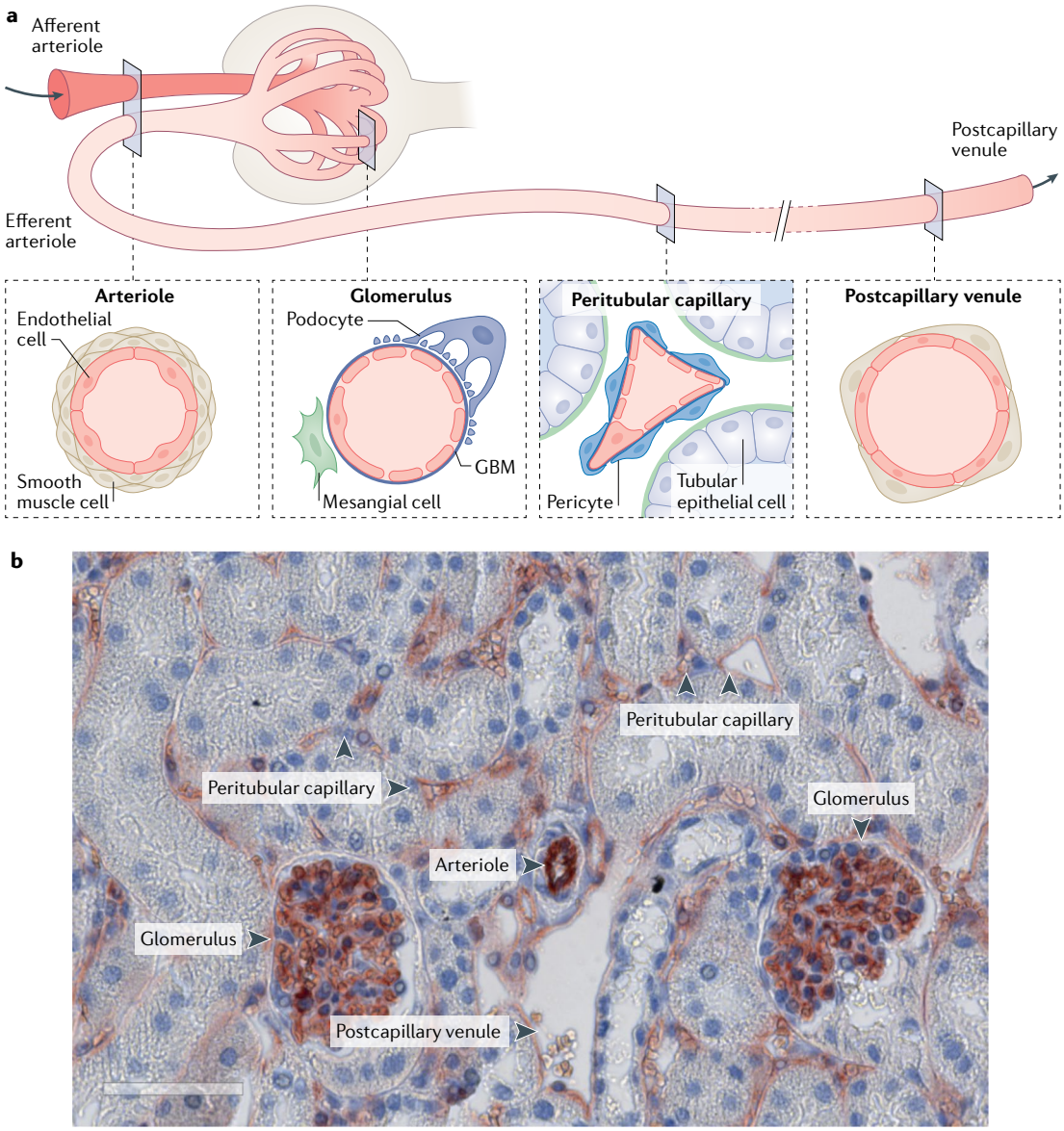

Fig. 1 | **Schematic and histology of mouse kidney cortex microvascular segments. a** | The microvasculature in the kidney cortex is made up of afferent arterioles that direct the blood into the glomerulus and efferent arterioles that transport the blood from the glomerulus into the peritubular capillaries, which surround the tubules. After traversing the medulla (not shown), the peritubular capillaries return to the cortex, where they deliver the blood into postcapillary venules. The endothelial cells in the microvascular segments of the cortex are surrounded by support cells: smooth muscle cells in arterioles and postcapillary venules, podocytes and mesangial cells in the glomerulus and pericytes in peritubular capillaries. Arrows show the direction of blood flow. **b** | Immunohistochemical staining of endothelial marker protein PECAM1 (also known as CD31) in formalin-fixed, paraffin-embedded mouse kidney cortex. The different microvascular segments of the cortex can be identified. GBM, glomerular basement membrane. Microscopy image courtesy of Dr Zhendong Wang, University Medical Center Groningen.

Kinases
Enzymes that phosphorylate target proteins in the signal transduction cascade, leading to activation of transcription factors and other molecular entities that can change the phenotype of a cell.

Signal transduction
The molecular mechanisms that are used by cells to relay an external stimulus into the nucleus to enable the necessary genes to be transcribed to mount a response.

Evans Blue Dye accumulation
An in vivo technique to assess organ microvascular permeability based on intravenous administration of this dye, which non-covalently associates with albumin.

Weibel–Palade bodies
Storage granules for P-selectin, angiopoietin 2 and von Willebrand factor proteins that are specifically located in endothelial cells.

shear stress affect the phosphorylation status of kinases and the expression and activation of transcription factors[34,35]. Shear stress can also affect the redistribution of proteins, as has been shown for vascular endothelial protein tyrosine phosphatase (VE-PTP; also known as PTPRβ)[36]. This intrinsic molecular heterogeneity of endothelial cells thus encompasses cell membrane components as well as molecules required for intracellular processes and signal transduction in response to external stimuli (TABLE 1, Supplementary Table 1). As the microenvironment influences endothelial cells in a multifaceted manner, it is unsurprising that endothelial cell cultures do not recapitulate their in vivo phenotype[15,37,38].

Consideration of microenvironmentally controlled endothelial phenotypes is important because molecular pathways in one microvascular segment might be absent in a different segment. This heterogeneity prevents simple extrapolation of observations from one vessel to another. Furthermore, differences in molecular responses of endothelial cells to disease stimuli, both within and between organs[15,39], have important implications for the identification of molecular targets for therapeutic interventions. Understanding of specific endothelial responses in sepsis-AKI may facilitate identification of (sets of) biomarkers that represent engagement of the microvasculature in the kidney instead of general endothelial activation. As the available data suggest that cortical changes underlie the acute injury seen in sepsis-AKI[40], in this Review we focus mainly on endothelial responses in the kidney cortex. When the relevant data are available, we also provide information about which changes occur in which microvascular segments.

Models of sepsis-AKI

The animal models that are most extensively used in sepsis-AKI research are endotoxaemia created by intraperitoneal injection of lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, and sepsis induced by caecal ligation and puncture (CLP). In both models, an inflammatory response occurs with an initial reduction in body temperature and elevation of systemic levels of cytokines[41–43]. At the same time, haemodynamic parameters change, GFR decreases and kidney function deteriorates, which is reflected by a temporary increase in blood urea nitrogen (BUN) and SCr levels and the occurrence of albuminuria and oliguria. Microvascular permeability (often assessed by Evans Blue Dye accumulation in tissues) increases and leukocytes are recruited.

In the endotoxaemia model, the severity and duration of loss of kidney function are highly dependent on the LPS serotype, dose and administration route, as well as the age and sex of the animal[41,44–46]. In the CLP-AKI model, release of bacterial content from the gut into the peritoneum marks the start of sepsis development and is followed by a multitude of systemic (FIG. 2) and functional changes comparable with those described for endotoxaemia-AKI[43,45,47]. The surgical strategy chosen has a major effect on CLP-sepsis disease severity[48]. Particularly in the CLP model, significant variation in disease severity occurs within groups of animals that develop sepsis-AKI[49–51] and is often considered to be inconvenient, yet reflects clinical observations of subgroups of patients with different severities of disease[52].

In human endotoxaemia, low-dose endotoxin is intravenously infused into healthy volunteers[53]. The resulting endotoxaemia resembles some of the features of sepsis but is less severe and of shorter duration[54,55]. Body temperature and heart rate increase and mean arterial blood pressure decreases. The levels of pro-inflammatory cytokines peak rapidly, followed by an increase in anti-inflammatory IL-10 and the formation of thrombi and fibrin[56,57]. Increased levels of *N*-acetyl-β-D-glucosaminidase (NAG) in urine indicate tubular injury[58]. Pharmacological studies using a specific inhibitor of inducible NO synthase (iNOS) indicated that proximal tubular injury is likely mediated by this enzyme[59]. In patients with sepsis, systemic cytokine levels also change over time[60]. Blood and urine samples are useful as they contain soluble biomarkers of sepsis-AKI development and progression[61]; however, post-mortem kidney biopsy samples from patients are invaluable as they provide information on the real clinical condition. The pathophysiology of human and murine sepsis has previously been reviewed in detail[62].

Endothelial responses in sepsis

Endothelial cells are one of the first cell types to sense changes induced by sepsis and undergo extensive molecular adaptations[63]. They employ biomechanical sensing properties to mount a rapid and specific reaction to changes in blood flow and blood pressure[64]. Furthermore, endothelial cells express receptors for cytokines, chemokines, damage-associated molecular patterns and pathogen-associated molecular patterns, including Toll-like receptor 3 (TLR3) and TLR4. Activation of these receptors leads to intracellular signal transduction and subsequent changes in cell phenotype[65,66] (FIG. 3). Changes in renal blood flow and renal capillary perfusion occur, with small diameter capillaries becoming physically blocked by thrombi. Blood vessel permeability increases leading to vascular leakage, leukocyte trafficking is induced and widespread molecular changes occur in the tissue[14,15,67].

Rapid responses are executed by almost instant release of proteins such as P-selectin, von Willebrand Factor (vWF), and ANG2 from Weibel–Palade bodies. In parallel, the expression of inflammatory adhesion molecules such as E-selectin, vascular cell adhesion protein (VCAM1) and intercellular adhesion molecule 1 (ICAM1), and of inflammatory cytokines and chemokines, including IL-6, IL-8 and monocyte chemoattractant protein 1 (MCP1; also known as CCL2), increases to guide leukocyte recruitment. Endothelial cell adhesion molecules in small diameter capillaries in which the architecture of the blood vessels dictates direct contact between white blood cells and endothelial cells might have roles in both 'inside-out' and 'outside-in' signal transduction in these cells[68–71].

Changes in cell–cell contact between endothelial cells that enable enhanced passage of leukocytes are coordinated by a complex molecular interplay between VEGF, VEGFR2, angiopoietins, TIE2, VE-cadherin

Table 1 | **Microvascular segment-specific expression of molecules with roles in endothelial function in the kidney cortex**

Molecule	Function	Species	Expression levels[a]				Refs
			Arterioles	Glomeruli	Peritubular capillaries	Postcapillary venules and veins	
Cx37	Cell-to-cell communication	Mouse	a+/e–	–	NA	NA	219
Cx40	Cell-to-cell communication	Mouse	a+/e–	–/+	NA	NA	219,220
		Rat	a+/e–	–	NA	NA	221
Cx43	Cell-to-cell communication	Mouse	a+/e+	–	NA	NA	219,220
ACKR1	Leukocyte recruitment	Human	–	–	–	+	222
		Mouse	–	–	–	+	223
EHD3	Endocytic vesicle component	Mouse	–	+	–	–	224
Endomucin	Leukocyte recruitment	Human	NA	+	+	+	225
eNOS	NO generation and/or signal transduction	Human	+	+	+	+	226
		Rat	++	–/+	–	–	101
EPCR	Coagulation	Human	–/+	–	–	+	140
ET1	Vasoconstriction	Human	+	+	–	–	227
ETB	Endothelin 1 receptor	Rat	–	+	+	–	92
Heparanase	Glycocalyx degradation	Mouse	+	+	–	–	132
IGFBP5	Proliferation, migration, inflammation and fibrosis	Mouse	–	+	+	–	228
MHC class II	Antigen presentation	Human	–	+	+	–	229
P2X7	Purinergic responses	Rat	a+/e–	+	–	–	230
S1PR1	Vascular permeability	Human	NA	++	+	–/+	231
TFPI	Coagulation	Human	–	–	+	–	232
		Rabbit	++	++	–/+	–	143
TNFR1	TNF signal transduction	Human	–/+	+	+	–/+	233,234
		Mouse	–	++	–	–	235
TIE2	Vessel stabilization	Human	NA	++	+	+	114
		Mouse	++	++	+	+	39,114
TM	Coagulation	Rabbit	NA	+	+	+	236
VEGFR1	Vessel stabilization	Human	–	+	+	–	237
		Mouse	–/+	–/+	++	–/+	238
VEGFR2	Vessel stabilization	Human	–	+	+	–	237
		Mouse	–/+	++	–/+	–/+	238
		Mouse	–/+	++	NA	+	39,114
VE-PTP	Signal transduction	Mouse	+	+	–/+	–/+	239
vWF	Coagulation	Human	+	+	+	+	233
		Human	+	–	–/+	+	240
		Mouse	+	–/+	–	+	241
		Baboon	NA	++	–	NA	242

–, no expression; –/+, limited or patchy expression; +, expressed; ++, highly expressed; a, afferent; ACKR1, atypical chemokine receptor 1; Cx, connexin; e, efferent; EHD3, EH domain-containing protein 3; eNOS, endothelial NO synthase; EPCR, endothelial protein C receptor; ET1, endothelin 1; ETB, endothelin 1 receptor type B; IGFBP5, insulin-like growth factor-binding protein 5; MHC class II, major histocompatibility complex II; NA, not available; P2X7, P2X Purino receptor 7; S1PR1, sphingosine 1-phosphate receptor 1; TFPI, tissue factor pathway inhibitor; TIE2, tyrosine protein kinase receptor; TM, thrombomodulin; TNFR1, tumour necrosis factor receptor 1; VEGFR, vascular endothelial growth factor receptor; VEPTP, vascular endothelial protein tyrosine phosphatase; vWF, von Willebrand factor.
[a]The expression levels noted in this table are based on published immunohistochemical or immunofluorescence staining patterns and/or mRNA data for specific segments, as well as descriptions of expression levels provided by the authors of the studies cited. Annotations of different vessels reported by a single study can be compared with each other; however, annotations obtained from different studies should not be compared as techniques, protocols, and antibodies for visualizing the molecules might not be the same.

and junctional adhesion molecules, as well as intricate interactions between the endothelium and leukocytes. Many of these molecules also have roles in controlling microvascular permeability[72–74]. For example, activation of the endothelial TIE2–ANG system by interaction of the coagulation factor vWF with platelets has been shown to have a role in the prevention of leukocyte diapedesis-associated microvascular leakage[75].

These functions of endothelial cells are crucial for an effective response to tissue damage, as they direct cells of the immune system to the site of infection and/or damage and initiate tissue repair[76]. However, not all processes are executed in all microvascular segments. Leukocyte recruitment, for example, mainly takes place in postcapillary venules to prevent organ function from becoming compromised[77] and molecular changes in endothelial cells in response to disease vary depending on the microvascular segment. Furthermore, as sepsis is an exaggerated, deranged and sustained host response to an infection, simple translation of normal inflammatory endothelial responses to sepsis is not possible.

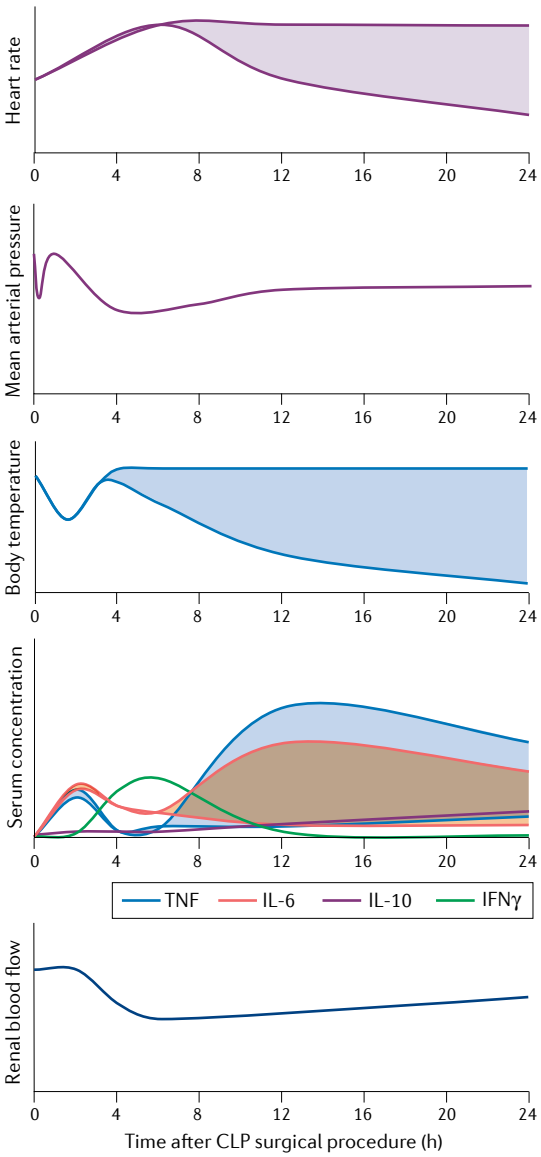

Fig. 2 | **Systemic and renal blood flow changes in mice with polymicrobial sepsis induced by CLP.** Polymicrobial sepsis initiated by caecal ligation and puncture (CLP) affects whole-body physiology, including haemodynamic parameters, circulating cytokine levels and local renal blood flow, in a time-dependent manner. The heart rate initially shows an increase whereas mean arterial pressure and body temperature fluctuate. Cytokines are produced soon after the start of CLP, resulting in a temporary rise in systemic concentrations with cytokine-specific kinetics. Renal blood flow starts to decrease 2–3 h after CLP and remains lower than baseline during the first 24 h of sepsis-AKI progression. All of these changes affect the behaviour of endothelial cells in the microvasculature of organs, including the kidney. The figure is based on data reported in previous studies[43,49,51,243–245]. The filled zones represent reported variations in responses of individual mice in a single experimental group[49,51]. This variation is similar to that seen among patients with sepsis[52,246]. IFNγ: interferon-γ; TNF, tumour necrosis factor.

Changes in blood flow and perfusion. Both hyperdynamic and hypodynamic circulatory conditions can affect GFR and kidney function[78]. Although consensus is lacking regarding the effects of experimental sepsis on renal macrocirculatory responses[47,79], insufficient renal microcirculatory perfusion leading to cortex hypoxia is generally believed to be associated with the development of sepsis-AKI[80]. A change in interplay between the afferent and efferent arterioles that control renal blood flow into and out of the glomerulus by controlling glomerular resistance likely causes loss of GFR[81]. In mouse endotoxaemia, GFR decreased within the first 2–4 h after LPS challenge and remained 80% lower than normal for up to 16 h (REF.[82]). Kidney function also rapidly deteriorated in mouse endotoxaemia, with BUN and SCr levels increasing 3–4-fold compared with healthy controls and only returning to basal levels 48 h after LPS exposure[83]. Changes in renal blood flow, accompanying hydrostatic pressure in the glomerulus and the permeability of the glomerular filtration barrier likely account for the decrease in urine production that occurs in endotoxaemia[84]. In addition, lower tubular flow rate and filtrate leakage from the tubular lumen into the interstitial space likely contribute to oliguria[85,86].

A hallmark of endotoxaemia is arterial vascular hyporesponsiveness to vasoconstrictors such as angiotensin II and noradrenaline (also known as norepinephrine). However, renal microvascular responses to these substances were normal or even enhanced in endotoxaemia-AKI[87]. In a live bacteria sepsis model in pigs, the kidney expression of angiotensin II type 2 receptor (AT2R) was upregulated more than 8-fold in those that developed AKI[50]. In addition, loss of AT1R has been reported in renal arterioles early after CLP and in glomeruli at a later stage of disease, with loss predominantly from the non-endothelial compartment[88]. As α-adrenergic receptors are mainly expressed by SMCs in arterioles and mesangial cells in glomeruli[89,90], responses of the renal microvasculature to changes in concentrations of vasopressors such as angiotensin II and noradrenaline during sepsis are likely to be of non-endothelial cell origin. Elevated plasma levels of endothelin 1 (ET1) occur early in endotoxaemia-AKI[91] and likely affect glomerular capillary pressure and GFR via ET1 receptors on vascular SMCs in arterioles[92,93].

A pharmacological study in endotoxaemic mice identified a role for iNOS in the loss of perfusion of cortical peritubular vessels and associated kidney function[94]. Early loss was likely a result of tubular epithelial cells responding to local general redox stress in the first few hours after LPS administration[83], with iNOS and other reactive nitrogen species having a later role[94,95].

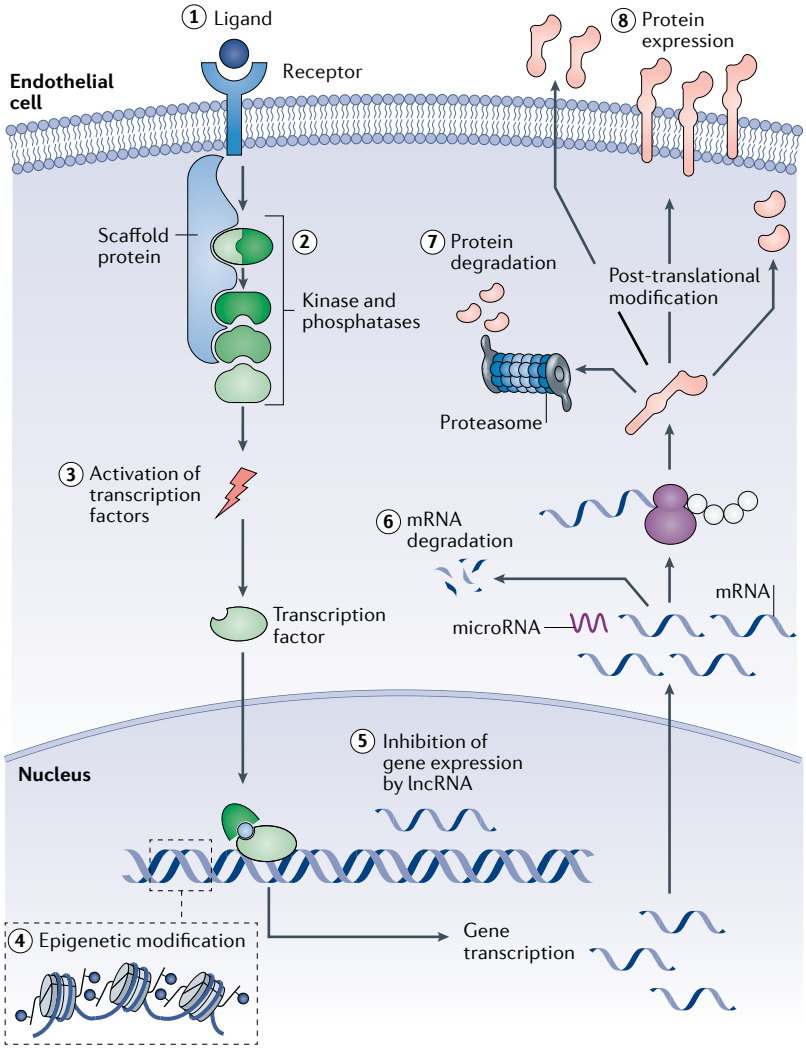

Fig. 3 | **Molecular pathways in endothelial cells that lead to phenotypic changes in response to sepsis conditions.** Endothelial cells can relay signals from the external milieu via receptors on the cell surface into the nucleus, driving gene transcription and subsequent protein expression, which lead to phenotypic changes. Several steps in this response can be employed as targets for therapeutic intervention and/or as a basis for the identification of biomarkers of endothelial engagement in sepsis-induced acute kidney injury (sepsis-AKI) initiation and progression. Activation of a receptor by ligand (step 1) binding results in relay of the signal by intracellular molecules such as kinases and phosphatases (step 2), which are thought to physically find one another via molecular scaffolds. This signalling leads to activation and recruitment of transcription factors to the nucleus[247–249] (step 3). Epigenetic modification of DNA and/or histones determines whether the transcription factor complex can bind and initiate gene expression[250] (step 4). Long non-coding RNAs (lncRNAs) (step 5) and microRNAs (step 6) further regulate whether proteins are translated[251,252]. Post-translational modification further regulates protein expression by targeting proteins for degradation (step 7). Protein sorting determines whether proteins remain within the cell, localize to the cell membrane or are excreted[253] (step 8). For clarity, this figure only depicts receptor tyrosine kinase signal transduction. Other type of membrane receptors, molecular mechanosensors and intracellular receptors are also active in endothelial cells and may employ different signalling pathways at different sites in the cell. Adapted with permission from REF.[189], Thieme Medical Publishers.

Expression of iNOS in the renal cortex 18 h after exposure of mice to a medium dose of LPS was highly restricted to tubular epithelium, whereas it was restricted to the arterioles and macula densa in rats with low-dose endotoxaemia[83,96]. General redox stress might also be the cause of high levels of protein nitration in endothelial cells in the arcuate artery, arterioles and glomeruli, and might contribute to changes in endothelial function[97].

Similar to observations in endotoxaemia models, the number of perfused cortical peritubular capillaries in rodent CLP-AKI decreased by 50% early after the start of sepsis and remained low for at least 18 h (REFS[98,99]). A direct role for NO in the initial stages of perfusion loss in this model is unlikely because the plasma and kidney levels of nitrate and nitrite did not begin to increase until 5–10 h after the start of sepsis[83,100]. In mouse CLP, expression of iNOS 16 h after the start of sepsis was restricted to glomeruli and tubules[95].

Endothelial NO synthase (eNOS) is highly expressed by endothelial cells in the arteries and arterioles, and to a lesser extent by those in glomeruli[101]. In addition to having a role in artery and arteriolar vasodilation by making gaseous NO available to surrounding SMCs, eNOS engages in intracellular signal transduction initiated by TIE2, VEGFR2 and VE-cadherin[72,102,103]. However, the NO-generating capacity of eNOS is much smaller that of iNOS[104]. In eNOS-deficient mice, BUN levels following CLP-AKI were almost threefold higher than those in controls with normal eNOS[105], indicating that eNOS has a role in controlling kidney function in the first 12 h after sepsis and likely ensures maintenance of renal blood flow and GFR[106]. In rats, exposure to LPS led to a reduction in GFR that was mechanistically linked to reduced eNOS activity in glomeruli[107], whereas CLP resulted in a major decrease in renal eNOS protein expression at 24 h after surgery[108].

Vascular permeability. VEGFR2 is an important regulator of vascular permeability and endothelial monolayer integrity. Upon extracellular activation of VEGFR2 by VEGF or intracellular eNOS, VE-cadherin becomes phosphorylated, endocytosed and degraded, resulting in an increase in vascular permeability[72]. In endotoxaemic mice, plasma levels of VEGF increased for up to 24 h after LPS administration, whereas kidney VEGF levels decreased to half that of healthy kidneys. This reduction in local VEGF expression might be the cause of ultrastructural changes and accompanying damage in glomeruli, including a decrease in the density of endothelial fenestrae and an increase in the diameter of remaining fenestrae. These changes were dependent on tumour necrosis factor receptor superfamily member 1 A (TNFRSF1A; also known as TNFR1) and directly associated with an increase in BUN and urine albumin to creatinine ratio[109]. Systemic lowering of VEGF levels in mice with endotoxaemia resulted in an extensive reduction in microvascular permeability in the kidney, suggesting a role for VEGF and VEGFRs in controlling this permeability[110,111]. In CLP, plasma levels of VEGF peaked 6 h after start at a lower level than seen in endotoxaemia and remained elevated at that level for up to 24 h (REF.[110]), with no change in kidney VEGF levels compared with controls[108]. As the effects of anti-VEGF treatment on renal microvascular leakage in CLP were not reported[110,111], whether or not there are differences in the role of VEGF–VEGFR2 in endothelial engagement in sepsis-AKI between CLP and endotoxaemia models is unknown.

Under stress conditions, such as those that occur in sepsis, a rapid increase in ANG2 plasma levels indicates extensive endothelial activation leading to release of pre-formed ANG2 from Weibel–Palade bodies[112]. The increase in ANG2 tips the ANG1 to ANG2 balance towards ANG2, leading to de-phosphorylation of TIE2, most likely via VE-PTP, and vessel destabilization[113]. In mice, renal levels of *Tie2* and *Ang1* decreased within 4h of exposure to LPS, whereas *Ang2* expression increased[114,115]. LPS administration also led to a temporary decrease in *Tie2* mRNA levels in major organs including the kidney, which might be explained by a decrease in renal blood flow[116] and/or epigenetic changes that lead to a decrease in RNA polymerase II binding sites in the DNA that encodes Tie2 (REF.[117]). Depletion of neutrophils prior to LPS challenge in mice reduced proteinuria but loss of Tie2 persisted, suggesting that Tie2 is not the only factor that controls glomerular barrier function[114]. Studies in mice with CLP sepsis-AKI showed that pretreatment with vasculotide, a PEGylated Tie2 agonist, protected against increased peritoneal permeability and improved renal function[118]. Treatment with recombinant human ANG1 immediately after the CLP procedure also protected against increased pulmonary permeability and improved renal function[119]. The effects of these treatments on renal microvascular permeability were not reported.

In endotoxaemic mice, pretreatment with COMP-ANG1, an engineered ANG1 construct that induces TIE2 phosphorylation, improved inulin clearance and GFR and attenuated the increase in microvascular permeability compared with controls[120]. As endothelial cells in the different microvascular segments in the kidney express similar levels of *Tie2*, local differences in expression of *Ang1* and *Ang2* might underlie local variations in engagement in response to sepsis conditions[39]. Further studies in endotoxaemia and CLP-AKI are needed to establish the roles of TIE2 and ANG in renal microvascular permeability control, via which mechanism(s) these roles are accomplished, and how they affect kidney function.

Sphingosine 1-phosphate receptor 1 (S1PR1) is a G protein coupled receptor that is abundantly expressed by endothelial cells[121]. Binding of sphingosine 1-phosphate (S1P) to S1PR1 leads to enhanced endothelial permeability barrier function via cytoskeletal rearrangements and adherens junction formation, whereas S1PR2 activation has the opposite effect[122]. S1PR1 also inhibits VEGFR2 signal transduction and consequently enables stabilization of VE-cadherin to maintain integrity of the blood vessels[123]. In endotoxaemia, plasma levels of S1P decreased by more than 50% within 24 h of exposure to LPS[124]. Treatment of mice with an S1PR1 agonist prior to the start of CLP resulted in a dose-dependent reduction in microvascular permeability 6 h after CLP and peritubular capillary perfusion remained compromised until full recovery at 18 h after CLP in these animals[99]. A study that used an *S1pr2*-knockout mouse model exposed to LPS identified a role of S1PR2 in renal microvascular permeability control[125]. However, this receptor is not extensively expressed by endothelial cells.

VE-cadherin degradation occurs in response to VEGFR2 activation, changes in shear stress and exposure of endothelial cells to inflammatory stimuli such as histamine and bradykinin[21,126,127], and leads to loss of endothelial adherens junction integrity and an increase in blood vessel permeability. In mice, low-dose LPS (0.5 mg/kg) induced a significant increase in VE-cadherin gene expression in the kidney, with no change in protein levels 4–24 h after the start of endotoxaemia[115], whereas in rats higher doses of LPS (14 mg/kg) induced rapid loss of VE-cadherin protein in glomerular microvessels within 4 h (REF.[128]). In one of the few studies to investigate renal function loss in CLP mice following the acute phase of inflammation, partial VE-cadherin protein loss persisted 30 h after CLP. At this time point, microvascular leakage no longer occurred[129], indicating that other junctional molecules had likely taken over microvascular permeability control.

In all sepsis models, endothelial glycocalyx loss rapidly results in elevated levels of soluble glycocalyx components in the blood. In mice, syndecan 1 plasma levels started to increase within 6 h and peaked around 24 h after high-dose intraperitoneal LPS administration (20 mg/kg). Electron microscopy analysis showed that 48 h after LPS administration, the glycocalyx had started to disappear from glomerular endothelial cells and podocytes and that endothelial fenestrae loss and swelling and gap formation between podocytes and basement membrane had occurred[27]. 24–48 h after LPS administration, increased renal heparanase expression was localized mainly in glomeruli. This increase was associated with glomerular loss of heparan sulfate glycosaminoglycans and functionally linked to increased BUN, elevated urine albumin:creatinine ratio, and glomerular injury[109,130]. In rat CLP-AKI, early glomerular syndecan 1 loss followed by hyaluronan loss at 7 h after CLP start was accompanied by modifications in glomerular sialic acids and molecular composition of the glomerular filtration barrier. Although no changes in SCr and creatinine clearance were seen, urinary albumin:creatinine ratio increased, indicating loss of glomerular filtration barrier permselectivity rather than of kidney function in general[131]. In mouse CLP, the expression and activity of heparanase increased during the first 4 h of AKI development, most prominently in the glomeruli and peri-glomerular arterioles. The accompanying loss of heparan sulfate partly accounted for the increase in BUN and fully accounted for the decrease in GFR induced by CLP[132]. An increase in renal microvascular permeability became apparent 8–24 h after CLP and showed a delayed response compared with the early rise in systemic levels of glycocalyx components[133].

Coagulation disbalance. One of the hallmarks of sepsis is disseminated intravascular coagulation, which results from an imbalance in anticoagulant and procoagulant processes[134]. In rat endotoxaemia, renal levels of pro-coagulant factors, including thrombin, fibrin and tissue factor (TF), increased during the first 1–3 h, whereas fibrinogen levels decreased[135]. Fibrin deposits were visible in the glomeruli and tunicae of arterioles within 3 h, even though endothelial cells in all microvascular segments upregulated their expression of proteinase-activated receptor 2 (PAR2), the receptor for the TF–Factor VIIa–Factor Xa complex, within this time

period[128,135]. In mice exposed to LPS, fibrin deposits were present within 6 h in glomeruli, peritubular capillaries, and postcapillary venules, and remained visible in these microvessels until 24 h after start of endotoxaemia[136]. Pharmacological blockade of PAR2 in a rat endotoxaemia model attenuated renal pro-coagulant status and therefore reduced fibrin deposits in the glomeruli but did not improve renal function[135]. This lack of improvement might be explained by other detrimental processes that interfere with kidney function such as a change in glomerular perfusion pressure.

Activated protein C (APC) interacts with EPCR and TM to protect blood vessel barrier function, both at the level of glomerular permeability and general microvascular permeability control[46,137]. An initial phase III trial of recombinant APC in patients with sepsis showed favourable outcomes; however, this treatment was removed from the market because subsequent trials failed to show beneficial effects[138]. Studies in experimental models of sepsis-AKI showed that EPCR has a role in creating an anti-coagulant state by promoting the activation of protein C by the thrombin–TM complex[137]. Shedding of EPCR and TM from endothelial cells disrupts the formation of this complex, resulting in a pro-coagulant state.

In endotoxaemia in mice and CLP in rats, kidney EPCR and TM protein levels decreased more than 50% within 6–12 h of initiation of sepsis[136,139], with loss of EPCR likely occurring in cortical arteries, arterioles and veins[140]. This decrease was paralleled by an increase in circulating soluble EPCR and TM levels and a decrease in plasma APC[136,139]. In mice, 6 h after LPS administration TF protein expression increased mainly in glomeruli, whereas fibrin depositions were visible in all microvessels. Compensatory transcription of genes encoding EPCR and TM increased in the same time period[136]. On the other hand, in rats, 12 h after start of CLP, microthrombi had filled all glomeruli whereas transcription of EPCR and TM had decreased[139]. In rats, temporary loss of TM in peritubular capillary endothelial cells and deposition of fibrin in glomeruli and peritubular capillaries occurred 20–60 min after high-dose LPS administration (20 mg/kg)[141]. These findings suggest that procoagulant activity in the glomerulus might depend more on TF upregulation by both endothelial and non-endothelial cells than on TM[136,142]. In healthy rabbit kidneys, tissue factor pathway inhibitor (TFPI) was highly expressed by endothelial cells in the arterioles and glomeruli and to a lesser extent by those in the peritubular capillaries[143]. Whether loss of TFPI selectively occurs in glomeruli after exposure to LPS and contributes to a local TF versus TFPI disbalance is unknown[144].

Inflammation and leukocyte recruitment. One of the first endothelial adhesion molecules with a role in leukocyte attraction that becomes available in the acute phase of sepsis is P-selectin, which is stored in Weibel–Palade bodies. In rat endotoxaemia, P-selectin is present in arterioles, glomerular capillaries and peritubular capillaries within 5 min of intraperitoneal LPS administration[145]. In endotoxaemic mice, E-selectin expression early after exposure to low-dose LPS occurred in all microvascular segments, with glomerular expression being the most prominent, whereas VCAM1 was expressed by endothelial cells in all microvascular segments except the glomeruli[114,146]. Co-expression of E-selectin and VCAM1 was predominant in peritubular capillaries and postcapillary venules[147]. E-selectin and VCAM1 were expressed in all vessels 18 h after high-dose LPS administration[125]. In addition, endothelial cells in all microvascular compartments expressed ICAM1 18 h after LPS exposure[125], whereas at 48 h its expression was restricted to endothelial cells in arterioles and peritubular capillaries[148]. In a baboon model of sepsis created by infusion of live *E. coli*, E-selectin expression occurred during the first 6–8 h in endothelial cells in arterioles, glomeruli and peritubular capillaries[149].

In mice exposed to LPS, neutrophils were prominently associated with glomeruli, cortical peritubular capillaries and postcapillary venules within 4 h and accumulation of neutrophils was still visible in the cortex after 48 h (REFS[114,150–152]). Depletion of neutrophils prior to LPS exposure did not prevent the rise in BUN that was associated with loss of kidney function[148], although proteinuria was attenuated[114]. Following long-term (3–7 weeks) repeated exposure of mice to LPS, VCAM1 was expressed by mesangial cells in the glomeruli and ICAM1 was expressed in glomerular endothelial and mesangial cells and in peritubular capillary endothelium. At 9 weeks of exposure, sparse populations of neutrophils were localized in the glomeruli and foci of infiltrating leukocytes were visible in the tubulointerstitial region. Glomerular changes included increases in glomerular size, cellularity and extracellular matrix expansion[153].

Studies performed in the early 2000s showed that LPS mainly activates extrarenal TLR4, resulting in systemic and local production of TNF, which acts via TNFR1 to instigate renal function loss and tubular injury, resulting in high BUN levels[150,151]. Which endothelial cells are responsible for cytokine and chemokine production is largely unknown. In two studies, one in anaesthetized, mechanically ventilated pigs exposed to continuous infusion of LPS, and one in mice exposed to intraperitoneal LPS, TNF was shown to be expressed by endothelial and mesangial cells in glomeruli, IL-1β by arteriolar endothelium and non-endothelial cell types in glomeruli[154] and MCP1 in arteriolar endothelium, SMCs and glomeruli[125].

In mice, CLP resulted in increases in P-selectin and E-selectin expression predominantly in glomerular and peritubular capillary endothelial cells, in VCAM1 expression in arterioles and in ICAM1 expression in glomeruli and peritubular capillaries[119,155]. Expression of several adhesion molecules remained increased 28 h after the start of CLP and was concurrent with elevated levels of numerous pro-inflammatory and anti-inflammatory cytokines, although information about their (microvascular bed) location is lacking[14].

Intravital microscopy identified increased rolling and adhesion of neutrophils in the cortical postcapillary venules between 4 h and 8 h after the start of CLP[155], with maximum total kidney accumulation plateauing at 12 h and remaining high until 24 h (REF.[156]). Macrophage 1 antigen (MAC1)-positive macrophages were located in

the glomerular compartment, and to a lesser extent in peritubular capillaries, 24 h after CLP start[157]. Neutrophil accumulation in the kidney depended on endothelial P-selectin, E-selectin and their leukocyte integrin counterparts, as shown using blocking antibodies, and neutrophil depletion at the time of CLP start prevented renal damage and rescued kidney function at 48 h (REF.[155]). Additional research is needed to identify the exact roles of P-selectin, E-selectin and ICAM1 in leukocyte recruitment in CLP sepsis-AKI, as neutrophil rolling and adhesion was reported to take place in postcapillary venules, whereas induced expression of these adhesion molecules occurred in glomeruli and peritubular capillaries. Furthermore, in transgenic E-selectin and P-selectin knockout mice, renal function improvement measured at 24 h after the start of CLP happened in conjunction with the inhibition of the local production of CXC-chemokine ligand 1 (CXCL1), whereas renal neutrophil accumulation did not change compared with wild-type controls[158].

Blood vessel contact time in the peritubular capillaries of lymphocyte antigen 6c (Ly6C)^{hi} inflammatory monocytes increased 6 h after CLP start, and the accumulation of these cells was shown to protect the kidney against damage caused by sepsis in a CX_3C-chemokine receptor 1 (CX_3CR1)-dependent fashion[159]. In the lung, endothelin receptor type B (ETB) expressed by endothelial cells is involved in ET1-induced expression of CX_3C-chemokine ligand 1 (CX_3CL1; also known as fractalkine), the ligand of CX_3CR1, thereby spatially controlling recruitment of protective monocytes[160]. In the kidney ETB is primarily expressed by endothelial cells in glomerular and peritubular capillaries[92] and could potentially have a similar role in protective monocyte recruitment as in the lung. However, this hypothesis remains to be investigated.

A role of the ANG–TIE2 system in endothelial inflammatory adhesion molecule expression and leukocyte recruitment in sepsis was identified in heterozygous *Tie2*$^{+/-}$ mice. In these mice, expression of E-selectin and VCAM1 4 h after LPS administration was ~50% lower than in wild-type controls, and this diminished response was accompanied by reduced leukocyte accumulation, particularly in the peritubular capillary region[152]. However, in a CLP-AKI model, heterozygous *Ang2*$^{+/-}$ mice also showed reduced glomerular and peritubular VCAM1 expression and leukocyte recruitment and lower SCr levels compared with wild-type controls[161]. In addition, treatment of mice with vasculotide or recombinant human ANG1 to enhance TIE2 phosphorylation before or at the time of CLP induction, respectively, resulted in a reduction in adhesion molecule expression in the renal microvasculature[118,119]. These results might be explained by an inhibitory effect of TIE2 phosphorylation on the production of pro-inflammatory cytokines (including TNF and IL-6) by TIE2-expressing cells of non-endothelial origin[118]. Furthermore, if interactions between the VEGF–VEGFR and ANG–TIE2 systems control leukocyte adhesion in these models as has been reported in vitro[162], differences in VEGF responses between endotoxaemia-AKI and CLP-AKI may also help to explain the apparently contradictory results.

The effects of sepsis-associated loss of glycocalyx on leukocyte recruitment in the kidney are less clear than the effects on permeability changes. In response to LPS, pulmonary capillaries in mice rapidly lose glycocalyx heparan sulfate, which leads to an increase in neutrophil recruitment[163]. In mice 48 h after LPS administration, monocyte and macrophage accumulation but not glomerular influx of neutrophils was glycocalyx dependent[130]. In CLP, no relationship between the loss of heparan sulfate from glomerular and periglomerular arteriolar microvessels and neutrophil influx could be established[132]. However, in anti-glomerular basement membrane glomerulonephritis, neutrophil recruitment into glomeruli was dependent on loss of heparan sulfate[130,164]. These data indicate that in sepsis-AKI the glomerular endothelial glycocalyx has restricted control over the recruitment of immune cells. They also highlight the importance of avoiding extrapolation of data obtained in other organs to the kidney.

Current knowledge of the changes in kidney function parameters and the systemic and local factors that are associated with endothelial behaviour during AKI initiation and progression in LPS and CLP models is far from complete (Supplementary Fig. 1). Moreover, further studies are required to delineate the spatiotemporal engagement of the renal microvascular endothelium in the initiation and progression of sepsis-AKI.

Endothelial cells in human sepsis

Numerous studies in human endotoxaemia and sepsis have reported changes in the systemic levels of molecules expressed by endothelial cells or used by receptors on endothelial cells that are involved in the processes underlying kidney dysfunction and injury. In volunteers who received LPS, plasma levels of VEGF and ANG2 increased within 4 h, whereas levels of ANG1 and soluble TIE2 did not change[110,112]. Among the inflammatory adhesion molecules, levels of soluble E-selectin increased within 2–4 h of the start of endotoxaemia and were followed by increases in soluble P-selectin, soluble VCAM1 and soluble ICAM1. All of these changes happened in the absence of clinical signs of kidney injury[112,165,166]. Plasma levels of the endothelial glycocalyx constituent endocan also rose rapidly in human endotoxaemia, returning to basal levels within 6 h of LPS administration[165].

In patients with sepsis, elevated systemic levels of ET1, NO-derived nitrite and nitrate species (NOx)[167–169], VEGFR1 (REF.[170]) and ANG2 (REFS[112,161]), as well as increased ANG2 to ANG1 ratio and decreased ANG1 to TIE2 ratio[171,172], were associated with disease severity. Plasma biomarkers can be used to help to subphenotype patients with sepsis-AKI into biologically distinct groups with different clinical outcomes and responses to therapy. The plasma ANG2 to ANG1 ratio was reported to be an important denominator for such subtyping[173]. Elevated NOx in patients with sepsis was associated with haemodynamic failure and mortality[169]. Serum levels of the haemostasis biomarkers vWF and TM were higher in non-survivors than in survivors, whereas VEGF and S1P levels were lower in non-survivors and inversely associated with disease severity[170,174]. Elevated levels of

soluble forms of the adhesion molecules VE-cadherin, PECAM1, E-selectin, VCAM1 and ICAM1 were also associated with increased disease severity[175–177]. The observation that soluble VE-cadherin levels were higher in patients with non-pulmonary sepsis than in those with pulmonary sepsis[177] suggests organ differences in VE-cadherin processing in sepsis, which has also been reported in mouse endotoxaemia[115]. In patients with septic shock, high urinary concentrations of glycosaminoglycans, particularly chondroitin sulfate, in the first 24 h after diagnosis were associated with the development of AKI within the next 24–72 h and with increased severity of illness[178]. Similarly, high plasma levels of syndecan 1 in patients undergoing resuscitation for severe sepsis or septic shock were associated with the development of sepsis-AKI[179]. The organ(s) and/or vasculature(s) in which the molecules that circulate at increased levels in sepsis are produced is not known.

A 2020 study made a valuable attempt to establish evidence to functionally link soluble endothelial markers, which in theory can be derived from any organ, with AKI in patients with sepsis. Plasma levels of cytokines, chemokines, soluble adhesion molecules, ANG1, ANG2 and blood coagulation activation markers, as well as blood leukocyte transcriptomes, were determined at admission and on day 2 and day 4 of hospitalization. The molecular data were associated with SCr levels to classify patients as having either transient or persistent AKI. The circulating levels of ANG1 and ANG2 provided discriminative power to differentiate between transient AKI and persistent AKI, which was associated with increased mortality. Patients who developed persistent AKI presented at admission with lower ANG1 and higher ANG2 levels than patients who developed transient AKI and patients who were admitted to the ICU for non-infectious disease[180].

In histopathological studies of kidney biopsy samples from patients with sepsis-AKI, 22% of samples showed acute tubular necrosis, with tubular cell apoptosis, which was also reported to occur in animal models, possibly resulting from local inflammatory processes and oxidative stress[10]. Post-mortem biopsy samples that were obtained from patients with sepsis-AKI immediately after death showed elevated monocyte and macrophage infiltration in glomeruli and peritubular capillaries. Neutrophils also accumulated in these microvascular segments, although the extent of infiltration was less than that of monocytes and macrophages[181]. A subsequent study confirmed preferential recruitment of these cell types, and found higher numbers of neutrophils in the tubulointerstitium, with no difference in T lymphocytes and B lymphocytes in post-mortem kidney biopsy samples from patients with sepsis-AKI compared with control samples from patients who underwent nephrectomy owing to renal cell carcinoma[182]. Macrophages that infiltrated into glomeruli were predominantly of the M2 type, which is associated with suppression of inflammation and enhanced repair[182,183].

Investigations into the expression of molecules involved in microvascular endothelial behaviour identified a decrease in the local balance of the ANG1 to ANG2 mRNA ratio in post-mortem kidney biopsy samples from patients with sepsis-AKI similar to that observed in animal models. Local expression levels of VEGF, VEGFR2 and junctional molecules also differed significantly from those in control kidney biopsy samples[115]. Another study showed that the expression of AT1R was lower in large resistance vessels, arterioles and glomeruli in post-mortem kidney biopsy samples from patients with sepsis compared with those from critically ill patients without infections. This reduction in AT1R expression might underlie reduced angiotensin signalling in these patients[88].

From molecule to function

Knowledge of endothelial engagement in sepsis-AKI remains incomplete. Not all of the molecules that are known to have roles in endothelial function have been investigated in detail in the kidney microvasculature. Furthermore, expression level changes in response to sepsis conditions are often only described at a whole organ level or in only one microvascular segment. The understanding of sepsis-AKI pathophysiology is lagging behind that of ischaemia–reperfusion injury, in which a wealth of information has been generated about cellular responses, including communication between tubular epithelium and peritubular capillaries and the microvascular dropout that precedes the development of chronic kidney disease[184,185].

Identifying which processes in which microvessels are directly related to the development of sepsis-AKI and kidney function loss, and could therefore be considered potential targets for pharmacological intervention, is a daunting task. Summarizing the main observations in the experimental models reviewed above might help to provide insights into how the microvasculature contributes to the control of perfusion, permeability, coagulant status, inflammation and kidney function, and its functional response to sepsis conditions.

Endothelial eNOS in arterioles and to a lesser extent in glomeruli has a role in renal perfusion changes in combination with an endothelial-independent, SMC-dependent vasopressor receptor functionality[19,89]. Rapid loss of peritubular capillary flow in sepsis-AKI likely results from tubular epithelial cell dysfunction associated with redox stress and might not represent a direct response of peritubular capillary endothelial cells to sepsis conditions[83]. In parallel, VEGF–VEGFR2 signalling controls vascular permeability in endotoxaemia, and in conjunction with TNFR1 signalling, is directly associated with glomerular damage and elevated BUN levels[109–111]. S1PR1 activation during CLP sepsis-AKI development inhibits an increase in microvascular permeability[99] but does not prevent a reduction in peritubular capillary perfusion during the first few hours of sepsis. In the endotoxaemia and CLP models of sepsis, the role of the TIE2–ANG system in proteinuria and kidney microvascular leakage seems limited[114,118–120].

The endothelial glycocalyx has multiple roles in kidney microvascular homeostasis. Intact levels of glomerular heparan sulfate are required for general kidney function and control of glomerular permeability[109,130], whereas heparan sulfate in arterioles is necessary for proper control of GFR[132]. Glomerular expression of

syndecan and hyaluronan contributes to a functioning glomerular filtration barrier[131]. Microvascular levels of these glycocalyx components change in a heterogeneous manner in sepsis-AKI, which, together with changes in other structural components of the endothelial surface layer, underlie microvascular function loss. Glycocalyx loss in glomeruli does not control neutrophil recruitment in sepsis-AKI models[130,132].

Fibrin deposits occur in all microvessels during the development of endotoxaemia-AKI and physically block blood flow[128,135,136]. The available data suggest that in the cortex, loss of EPCR likely happens in arterioles[140] and loss of TM in peritubular capillaries[136], whereas early TF expression is localized in the glomeruli[136]. This heterogeneous yet all-encompassing response can explain the occurrence of fibrin deposits in all microvascular segments and the absence of functional improvement when only one pathway is pharmacologically inhibited. Each microvascular segment could potentially have its own molecular mechanism(s) to control anti-coagulant versus procoagulant status.

Although arteriolar endothelial cells express inflammatory cell adhesion molecules in response to sepsis conditions, leukocytes do not extensively associate with the vessel wall in these microvascular segments[152]. The role of inflammatory cell adhesion molecule expression in arteriolar endothelial cells in loss of kidney function in sepsis-AKI remains enigmatic, as does the role of inflammatory adhesion molecule expression in glomerular and peritubular capillary endothelial cells, while leukocytes are being recruited in the postcapillary venules[155]. Whether functional interactions occur between leukocytes and endothelial cells in these small lumen blood vessels and whether such interactions could lead to further activation of both cell types prior to tethering and adhesion of the cells in the postcapillary venules remain to be established. Another remaining question is which molecular entities other than the commonly known adhesion molecules such as P-selectin, E-selectin, VCAM1 and ICAM1 are required for effective leukocyte recruitment. The role of the ANG–TIE2 system in leukocyte recruitment in experimental sepsis-AKI might entail a direct effect on endothelial cell signalling and subsequent changes in behaviour, or molecular changes in TIE2-expressing leukocytes, or a combination of both mechanisms[118,152].

Given the importance of the peritubular capillaries in normal kidney function, molecular changes in endothelial cells in this segment likely also contribute to kidney function loss in sepsis-AKI[28]. The observations that systemically administered LPS rapidly enters the tubular lumen after being filtered in glomeruli[186] and that local application of bacteria into proximal tubules leads to coagulation and shutdown of blood flow in the peritubular capillaries directly adjacent to the infected tubules[187,188] necessitate further investigations into the occurrence, molecular nature and functional consequences of communication between tubular epithelial cells and peritubular capillary endothelial cells in sepsis-AKI. Furthermore, as supporting pericytes extensively express vascular homeostasis genes such as VEGF and angiopoietins[28], we speculate that many of the molecular systems discussed above might also contribute to peritubular capillary engagement in the development of sepsis-AKI. However, further studies are required to investigate this hypothesis.

Mechanisms of heterogeneous responses

Understanding of the molecular mechanisms by which endothelial cells respond to sepsis-AKI conditions will provide a rational basis for the development of pharmacological interventions[189]. In vitro studies using endothelial cells exposed to conditions that mimic sepsis-AKI have provided highly detailed information about these processes and identified roles of nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)–AKT signalling and Rho kinases in control of the expression of inflammatory molecules, pro-thrombotic state and loss of monolayer integrity[65,190–192].

Only a few studies have followed up these findings in vivo. In transgenic mice that overexpressed a protein that specifically inhibits NF-κB activation in endothelial cells, LPS-induced expression of EPCR in the kidney was inhibited by up to 70% and endothelial cell EPCR protein shedding was reduced compared to control mice. Lack of endothelial NF-κB signalling capacity also ameliorated the loss of TM protein in response to endotoxaemia and resulted in a decrease in endothelial TF expression in glomeruli. As a result, fibrin deposition was almost completely absent in the transgenic mice. These data show that in mouse endotoxaemia, NF-κB signalling in kidney microvascular endothelial cells has an important role in creating conditions that favour a pro-coagulant state[136]. Congestion of glomerular and peritubular capillaries, increases in endothelial permeability and accumulation of neutrophils in mice with endotoxaemia were also dependent on endothelial NF-κB activation[193].

In the first 3 h after LPS administration in mice, activated Rho kinase was mainly localized in peritubular capillary endothelial cells; 6 h after LPS administration Rho kinase activation could also be detected in glomerular endothelium and podocytes[194]. Pharmacological inhibition of Rho kinase prior to LPS administration inhibited Rho kinase activity in endothelial cells in both microvascular segments. This effect was paralleled by lower BUN levels and reduced neutrophil, monocyte and macrophage influx. As systemic administration of Rho kinase inhibitors can theoretically also block Rho activity in leukocytes and tubular epithelial cells, further studies are needed to investigate the role of endothelial Rho signal transduction in kidney function loss and leukocyte recruitment in sepsis-AKI.

Epigenetic mechanisms that control access of transcription factors to the promoter regions of DNA encoding inflammatory molecules or vascular site-specific expression of molecules that compete with NF-κB transcriptional activity may explain local microvascular endothelial heterogeneity in response to disease conditions[195]. In the kidney, constitutive variation in expression of receptors such as TNFR1 and VEGFR2 (TABLE 1) could potentially drive heterogeneous responses seen in endotoxaemia and CLP-AKI (FIG. 4). In addition, variation in how endothelial cells in different

microvasculatures relay signals from receptors into the nucleus or process transcribed mRNA into protein could explain the heterogeneous response of endothelial cells to sepsis-AKI conditions. A complicating factor in uncovering the causes of the heterogeneous behaviour of these cells is that during disease development, changes occur in factors such as the expression of receptors involved in endothelial responses. For example, 24 h after challenge with LPS, TLR2 expression increased in glomerular endothelium and podocytes[196], whereas in CLP-AKI, TLR4 expression increased in glomeruli, at the vascular pole of glomeruli and in peritubular capillaries[197].

Future perspectives

Sepsis-AKI research is challenging because the disease involves complex pathophysiological processes in heterogeneous patients and many different cell types and molecular factors in the tissue and circulation that dynamically alter their behaviour in a poorly understood manner. In addition, by the time a critically ill patient is seen by a physician, several pathophysiological pathways will already have been activated in an unknown temporal fashion. As abundant evidence now exists of active involvement of endothelial cells in renal responses to sepsis in animal models, new studies aimed at utilizing these cells as targets for pharmacological intervention are expected. Use of individual therapeutic strategies such as halting change in arteriolar status, inhibiting increased permeability, preventing fibrin depositions and prohibiting inflammatory adhesion molecule expression are unlikely to be successful, as many other cell types and molecular processes contribute to sepsis-AKI pathophysiology[180,198–202]. However, improved understanding of how renal endothelial cells contribute to sepsis-AKI, in combination with knowledge of the contributions of other cell types, could potentially lead to new therapeutic strategies such as combination therapies or therapies that do not target a single signal transduction pathway.

To further sepsis-AKI endothelial biomedicine research, the spatiotemporal behaviour of renal endothelial cells needs to be studied in their natural niche[203,204]. A lot of information about microvascular endothelial responses to sepsis conditions and drug interventions could potentially be obtained from existing frozen tissue samples and microscope slides in laboratories worldwide. Investing time and effort in assessing each microvascular bed when analysing molecular determinants in the kidney is imperative. Moreover, the effects of ageing and comorbidities should be considered when

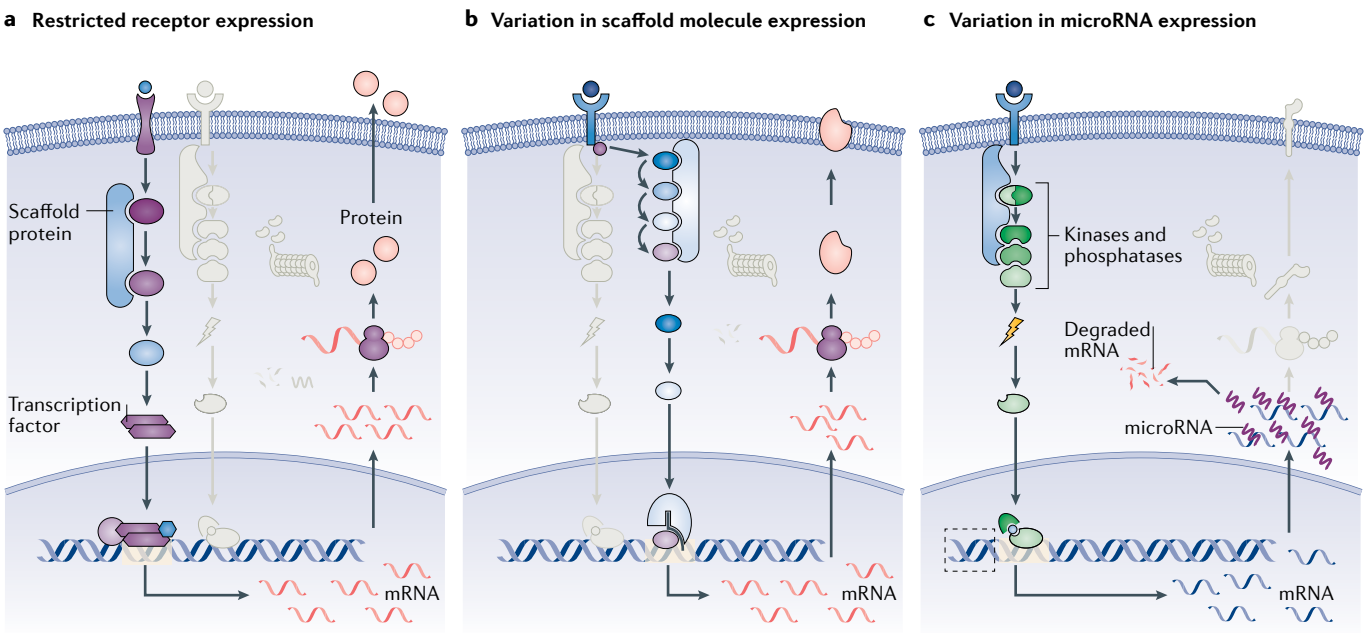

Fig. 4 | **Molecular mechanisms that could underlie renal microvascular endothelial cell heterogeneity in responses to sepsis-AKI.** Endothelial cells in the kidney microvasculature respond in a location-specific manner to disease-associated stimuli such as those that occur in in sepsis. These responses contribute to loss of kidney function, increased microvascular permeability, intravascular coagulation and recruitment of immune cells. Three molecular mechanisms likely contribute to the heterogeneity of endothelial cell responses. **a** | Restricted receptor expression could lead to segment-specific responses. For example, tumour necrosis factor receptor 1 is expressed by glomerular and peritubular capillary endothelial cells[233–235]. High levels of tumour necrosis factor that are systemically released in response to lipopolysaccharide administration or caecal ligation and puncture might preferentially activate these cells in the first few minutes or hours of sepsis-induced acute kidney injury (sepsis-AKI) development. **b** | Variation in scaffold protein expression could deviate the signal induced by a receptor towards activation of different transcription factors. For example, variation in scaffold protein expression in T lymphocytes was shown to result in activation of alternative signal transduction pathways and transcription factors[254]. This mechanism has not yet been demonstrated in endothelial cells in renal microvascular segments but could potentially contribute to their heterogeneous behaviour. **c** | Differences in microRNA expression between endothelial cells result in variation in post-translational processing of target mRNA. For example, limited VCAM1 protein expression in glomerular endothelial cells in response to systemic inflammation was associated with high glomerular expression of the microRNA-126, as well as high expression of *Egfl7*, which encodes miR126, and of the transcription factor Ets1, which controls miR126 expression[255]. In this figure, the greyed out signal transduction pathway represents the default pathway used by a cell.

Precision cut kidney slice (PCKS) technology
A technology that reproducibly cuts tissue slices of predetermined size from kidney biopsy samples or whole kidney tissues. This technique preserves tissue architecture and enables cellular responses to drugs and experimental conditions to be studied.

Laser microdissection
A technique that combines regular microscopy with laser-based release of selected parts of a tissue section for further molecular analyses.

Omics
The collective name for platform technologies that assess the molecular status of tissues and/or cells in an unbiased manner, that is, the analysis is not restricted to pre-selected molecules.

Pharmacolomics
The study of drug effects on target cells and surrounding tissues using omics platform technologies.

designing experimental studies in animals[205,206]. The use of omics platform technologies to investigate the transcriptomes and proteomes of endothelial cell subsets in the healthy kidney and in conditions that exist in sepsis[207–209] will be extremely helpful in deciphering their heterogeneity and the complexity of their responses (Supplementary Table 1).

A major challenge in identifying the molecular identity of endothelial cells in microvascular segments is to avoid compromising the histopathology, location information and molecular status of these cells. Precision cut kidney slice (PCKS) technology retains tissue architecture and can be used to expose endothelial cells in their local niche to conditions that prevail in sepsis. This technology is a valuable experimental tool that can be used for animal and human samples and as such bridge the translational gap[210,211]. Furthermore, consecutive pharmacological studies can be performed using PCKS from a single rodent or large animal with sepsis-AKI, thereby supporting the 'Reduce, Refine, and Replace' principles in biomedicine.

Laser microdissection of segments from tissue sections in which the biomolecular status is retained, followed by analysis using omics platform technologies, can enable detailed molecular information to be obtained[212–214]. This strategy can be applied to kidney tissue from experimental animals and post-mortem kidney biopsy samples from patients with sepsis, as well as to PCKS. The identification of the endothelial signatures of different clinical subtypes of AKI, in combination with the retrieval of extensive clinical and biochemical data, could potentially lead to the identification of kidney-specific biomarkers and the development of precision medicine approaches for sepsis-AKI[173,180,215,216].

The use of organ-on-chip designs and organoid cultures have gained momentum in biomedicine. Extensive effort is being focused on the improvement of kidney organoids to overcome limited vascularization and limited vasculature maturation[217,218]. A mature vasculature is a key requirement for the application of organoids to the study of microvascular endothelial responses to sepsis conditions and the design of drug intervention strategies.

Numerous drugs that interfere with molecular systems in endothelial cells, for example, the VEGF–VEGFR2 and ANG–TIE2 systems, are already in development for various diseases and could potentially be repurposed for the treatment of sepsis-AKI. However, such approaches will require knowledge of the molecular status of these and other signalling receptors in the kidney and other organs, as well as whether this status changes during sepsis-AKI development, to reduce the risk of adverse effects. The effects of these drugs on endothelial cells in different microvascular segments in the kidney during sepsis-AKI could be investigated using laser microdissection and pharmacolomics strategies. In particular, careful assessment of receptor phosphorylation and the effects of drug treatment on receptor phosphorylation status in tissue samples would be required. Such assessment in animal models using high-quality phospho-protein detection techniques could be related to functional parameters in the organs of interest to avoid wrongful extrapolation from observations in other organs.

Conclusions

Microvascular endothelial cells in the kidney are active contributors to the development of sepsis-AKI in animal models and these findings are supported by clinical data. Molecular and functional heterogeneity between endothelial cells in microvascular segments both within and between organs prevents extrapolation of observations from one organ or microvascular segment to another. Instead, these cells must be studied in the location in which they reside. A number of molecules associated with endothelial function have roles in loss of blood flow, increases in blood vessel permeability, blood coagulation, inflammatory cell recruitment and associated deterioration in kidney function in sepsis. Improved understanding of the engagement of endothelial cells in the complex processes that are associated with sepsis-AKI could lead to new therapeutic strategies. However, such progress will require careful choice of models and molecular technologies to study the engagement of these molecules in greater detail, interrogation of all microvascular segments in the kidney and greater availability of human kidney tissue, blood and urine samples, as well as patient data, for validation of experimental findings.

Published online: 19 October 2021

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Author contributions
G.M., J.G.Z. and M.v.M. researched the data and wrote the article. G.M., J.G.Z. and J.A.A.M.K. made substantial contributions to discussion of the content. All authors reviewed or edited the manuscript before submission.

Competing interests
J.G.Z., M.v.M. and J.A.A.M.K. declare no competing interests. G.M. is co-founder and chief technology/science officer of Vivomicx, which provides tissue laser microdissection and omics analysis services.

Peer review information
Nature Reviews Nephrology thanks the anonymous reviewers for their contribution to the peer review of this work.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41581-021-00489-1.