Novel Proinflammatory Function of Renal Intercalated Cells

Sylvie Breton · Dennis Brown

Center for Systems Biology, Program in Membrane Biology/Nephrology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Keywords

Kidney inflammation · UDP-glucose · Purinergic receptors · Kidney collecting duct · Neutrophil infiltration

Abstract

Background: Serious and often fatal acute kidney injury (AKI) is frequently seen after major surgery, local and remote organ damage, and sepsis. It is associated with uncontrolled inflammation, and is usually diagnosed only after the kidneys have gone through significant and often irreversible damage. **Summary:** During our work involving another type of kidney disease that leads to acid-base disorders of the blood, we unexpectedly found high levels of a protein called the P2Y₁₄ “purinergic” receptor, in specialized kidney epithelial cells called intercalated cells (ICs). These cells are responsible for maintaining whole body acid-base balance by regulating the secretion of excess protons into the urine, which normalizes blood pH. However, it turns out that the P2Y₁₄ receptor in these cells responds to a molecule called uridine diphosphate (UDP)-glucose, which is a danger signal released by damaged cells anywhere in the body. When UDP-glucose reaches the kidney, it stimulates ICs to produce chemoattractant cytokines; this results in renal inflam-

mation and contributes to the onset of AKI. **Key Message:** Thus, our work now points to ICs as key mediators of renal inflammation and AKI, following surgery and/or damage to remote organs, sepsis, and also local insults to the kidney itself. The link between the proton secreting ICs of the kidney and AKI is an example of how a fundamental research project with a defined aim, in this case understanding acid-base homeostasis, can lead to a novel observation that has unexpected but major implications in another area of human health.

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Introduction

Renal intercalated cells (ICs) are best known for their central role in the regulation and maintenance of acid-base balance via the proton-pumping activity of the vacuolar H⁺-ATPase (V-ATPase), and other membrane associated ion transporters. However, we recently made the unexpected discovery that in addition to this important physiological role, type A proton secreting ICs (A-ICs) are also sensors that mediate sterile inflammation

in the kidney medulla [1]. This resulted from our identification of high expression of the proinflammatory uridine diphosphate (UDP)-glucose receptor P2Y₁₄ in A-ICs.

Renal failure (i.e., in acute kidney injury [AKI] brought on by surgery, local and remote organ damage, and sepsis) is often associated with uncontrolled inflammation [2]. Prolonged episodes of dehydration also can result in AKI and chronic kidney disease (CKD) [3]. In addition, acidosis is a comorbidity and co-mortality factor in AKI [4]. In this context, we discuss here the well-established role of A-ICs in acid-base homeostasis, and the newly discovered proinflammatory role of these cells.

AKI – The Silent Killer

Regardless of the cause of hospitalization, all patients admitted to the intensive care unit (ICU) are at a high risk of developing AKI [5]. On a yearly basis, up to two thirds of the 5.7 million ICU patients in the United States develop AKI [6, 7]. Overall, AKI affects 1 in 5 hospitalized adults, and 1 in 3 hospitalized children [8]. In addition, patients who survive AKI have an increased chance of developing CKD and end-stage renal disease [9]. However, AKI often remains undetected due to the lack of adequate biomarkers of early renal injury, and very few therapeutic options are currently available to alleviate AKI [10, 11]. This leads to AKI sometimes being referred to as “the silent killer.” A recent review article stated, “Given the high mortality and morbidity associated with AKI, there is an important unmet medical need to develop effective therapies for these patients” [11]. In this context, the field is in dire need of a biomarker that can detect early signs of renal injury, and new treatments that could be administered while injury can be prevented. This would enable the physician to make vital clinical decisions before the damage progresses to potentially fatal renal failure.

Multiple primary clinical conditions are associated with AKI, especially cardiac surgery, myocardial infarction (MI) and sepsis. AKI occurs in 30% of patients after cardiac surgery and in 22% of patients after MI [12]. These patients are then at high risk of multiple organ failure and death. In addition, sepsis is responsible for over 50% of cases of AKI in the ICU [4, 11]. Thus, the means by which diverse organ systems communicate damage to the kidney, which leads to AKI, is of great current interest in the nephrology field.

Purinergic Signaling in the Kidney

Uncontrolled inflammation is a major cause of AKI [2]. Following immune cell chemotaxis and infiltration, production of reactive oxygen species, eicosanoids, and leukocyte- and endothelial cell-derived mediators create an intense inflammation reaction that potentiates renal injury [13, 14]. Purinergic signaling has been shown to participate in the onset of renal inflammation, but the underlying mechanisms involved, and how to prevent and alleviate the severe damage caused by inflammation, still remain to be fully characterized. Purinergic receptors respond to extracellular purines and pyrimidines, and they modulate a variety of cellular functions [15]. P2X receptors are ligand-gated ion channels and their principal selective agonist is adenosine triphosphate (ATP), while P2Y receptors are G protein-coupled receptors that respond to a variety of adenine and uracil nucleotides, including ATP, adenosine diphosphate (ADP), uridine triphosphate (UTP), UDP, and UDP-sugars. Several purinergic receptors are present along the renal tubule, and numerous pathologies are associated with dysregulation of purinergic signaling, including hypertension, CKD, AKI, diabetic nephropathy, and glomerulonephritis [16, 17]. Purinergic receptors present in the renal collecting duct principal cells regulate water, electrolyte, and volume homeostasis [18, 19]. We recently showed that the adenosine receptors ADORA2A and ADORA2B, located in the apical membrane of the collecting duct ICs, stimulate proton secretion via activation of the cAMP/PKA pathway [20]. In this review article, we describe how another member of the purinergic family, the proinflammatory receptor P2Y₁₄, mediates renal inflammation via ICs [1], and suggest how this might be applicable to the diagnosis and treatment of AKI.

Identification of the P2Y₁₄ Receptor in Collecting Duct ICs

The P2Y₁₄ receptor (also known as GPR105) is specifically activated by nucleotide sugars [21]. In contrast to other P2Y receptors, it is insensitive to ADP, ATP, and UTP, but its specific ligand has been identified as UDP-glucose. Extracellular ATP, adenosine, and UDP-glucose are immune-regulatory factors known as DAMPs (damage associated molecular pattern) molecules [22]. DAMPs initiate sterile inflammatory reactions, while PAMPs (pathogen associated molecular patterns) trigger infectious proinflammatory responses [22, 23]. UDP-glucose is released by

Fig. 1. Immunofluorescence localization of P2Y₁₄ in the apical membrane of type A intercalated cells (ICs). Double-labeling for the IC marker V-ATPase (red), and P2Y₁₄ (green) on a section of fixed kidney medulla using specific antibodies against these

proteins. P2Y₁₄ is expressed on the apical membrane (urinary side) of A-ICs, where it co-localizes with the V-ATPase (arrows and yellow in merge panel). Scale bar = 20 μ m.

injured cells and acts as an autocrine activator of the P2Y₁₄ receptor [24]. In contrast to most nucleotides, which are rapidly degraded by ectonucleotidases after their release, UDP-glucose resists hydrolysis by these enzymes [25].

P2Y₁₄ is an inflammatory mediator that is expressed in immune cells [26–28], the brain, gastrointestinal tract, kidney, and lungs [1, 21, 29]. Patients with cystic fibrosis and asthma have elevated concentrations of UDP-glucose in their lungs [30, 31], and P2Y₁₄ activation by UDP-glucose in airway epithelial cells leads to interleukin 8 secretion and an inflammatory response [32]. Furthermore, mouse uterus injection of UDP-glucose induces the recruitment of neutrophils into the endometrium [33]. In addition, P2Y₁₄ mRNA expression is upregulated by lipopolysaccharides, further indicating its role in mediating inflammation [33, 34].

Our recent screening of the proteome and transcriptome of ICs unexpectedly revealed very high expression levels of the purinergic receptor P2Y₁₄ [1]. ICs were isolated by fluorescence activated cell sorting from previously characterized transgenic mice that express enhanced green fluorescent protein (EGFP) specifically in these cells (B1-EGFP mice) [35]; no expression was detected in all other renal cell types [1]. In addition, RNA sequencing of isolated EGFP ICs identified P2Y₁₄ as one of the most expressed genes in these cells, while single cell RNA sequencing showed specific expression of P2Y₁₄ in type A ICs [36]. Subsequent examination showed that P2Y₁₄ is located on the apical surface of ICs in contact with the tubular fluid (pre-urine; Fig. 1).

The Novel Role of ICs in Renal Inflammation: Implications for the Onset of AKI

Treatment of mice with a single i.v. injection of UDP-G increased proinflammatory chemokine expression in ICs isolated 4 h later by fluorescence activated cell sorting from transgenic mice [1]. In cultured MDCK-C11 cells, which also express P2Y₁₄, UDP-G treatment increased ERK1/2 phosphorylation and upregulated chemokine mRNAs through the activation of P2Y₁₄ [1]. Flow cytometry analysis showed a robust renal neutrophil infiltration 2 days after UDP-G administration in mice, and neutrophils were often seen in dose proximity to ICs. We did not detect P2Y₁₄ in murine neutrophils, so it is unlikely that UDP-G acted directly on these cells to induce chemotaxis in an autocrine manner [1]. These results indicated a novel role for ICs as sensors, mediators, and effectors of sterile inflammation in the kidney via P2Y₁₄. We suggest that, by their ability to produce chemokines, ICs act as immune defense cells by creating a chemotactic gradient favorable to immune cell recruitment (Fig. 2).

This study enhanced our understanding of how a local insult to the kidney and also a distant injury to another organ are able to communicate damage to the kidney through the activation of the proinflammatory receptor P2Y₁₄ located in renal ICs. Our working hypothesis is that UDP-G (i.e., released from injured renal proximal tubules during ischemia reperfusion injury, cardiomyocytes during MI, or cells damaged in sepsis) is a circulating biological mediator

Fig. 2. Proposed mode of action of UDP-G in mediating inter- and intra-organ cross-talk resulting in renal inflammation. UDP-G is released from cells injured during myocardial infarction (MI), sepsis, or proximal tubule ischemic injury. UDP-G released from these injured cells either in remote organs such as the heart, or more locally in the kidney itself, is delivered to the lumen of the kidney collecting duct, where it binds to the P2Y₁₄ receptor located on the apical surface of A-ICs. This receptor-ligand interaction stimulates the production of proinflammatory chemokine (PICS) by ICs, which attract neutrophils from the circulation into the kidney.

that initiates intra- and inter-organ crosstalk and causes renal inflammation and AKI (Fig. 2). Why would UDP-G affect the kidneys more than any other organ in the body? One of the main roles of the kidneys is to remove metabolites such as UDP-G from the blood, while maintaining a constant blood volume. During this process, serum UDP-G is filtered by glomeruli and is delivered to the urinary compartment of the kidney. Upon water extraction from the collecting duct, by the aquaporin-dependent renal concentrating mechanism [37], UDP-G is then concentrated in the lumen of the collecting duct to a critical point where it initiates local inflammation through the activation of P2Y₁₄ located in the apical membrane of ICs [1].

Role of ICs in Acid-Base Regulation

ICs secrete protons and are located along the collecting duct of the kidney, where they are interspersed among the water-reabsorbing principal cells (Fig. 3). Up until the recent discovery of their immune function described above, they were best known as regulators of systemic acid-base balance. They survey the local environment and rapidly adjust the amount of a critical enzyme, the proton pump or V-ATPase, at the cell surface to control blood pH by expelling protons into the urine (Fig. 3) [38–41]. Interestingly, a subset of ICs can work in the reverse direction, by pumping protons into the blood and secreting bicarbonate into the tubule lumen. This process is activated when blood

Fig. 3. Immunofluorescence identification of different cell types in the collecting duct. Triple-labeling for V-ATPase (blue), aquaporin 2 (green) and aquaporin 4 (red) of fixed kidney medulla. A-ICs express the V-ATPase in their apical membrane (arrows), consistent with their function of secreting protons into the tubule lumen. The adjacent principal cells express apical aquaporin 2 (green) and basolateral aquaporin 4 (red), consistent with their role in water reabsorption from the tubule. Scale bar = 5 μ m.

pH becomes too alkaline [38]. One of the major pathways positively or negatively regulating the amount of active V-ATPase at the cell surface is the vesicular shuttling of V-ATPase molecules to and from the cell surface by exo- and

endocytosis of very unique V-ATPase-rich vesicles. Readers interested in this cell biological process are directed toward review articles on this topic [38, 41, 42].

While acid-base disturbances have multiple origins, deficient V-ATPase function results in acid buildup in the body, leading to acidemia and childhood-onset distal renal tubule acidosis. If undiagnosed and untreated, distal renal tubule acidosis may lead to failure to thrive, growth retardation, osteomalacia or rickets, nephrocalcinosis or nephrolithiasis, kidney failure, and even death in infancy [43]. The V-ATPase is a very complex enzyme formed of at least 13 different protein subunits [38]. Loss of function mutations in some of these subunits in mice and humans results in persistently high urine pH, hypobicarbonatemia, hypercalciuria, hypocitraturia, and hypokalemia, often with progressive sensorineural hearing loss [44] and olfactory defects in mice [45].

Thus, the majority of research directed toward these cells in the past has understandably focused on their central role in acid-base regulation. However, 2 previous reports also have implicated ICs in defending the urinary tract against bacterial infection through the Toll-like receptor-4-mediated innate immunity pathway, which recognizes urinary pathogens, in particular uropathogenic *Escherichia coli* [46, 47]. Together with our more recent data, a new function for ICs in defending against bacterial infection and mediating sterile inflammation is emerging.

Conclusion

The link between proton-secreting ICs of the kidney and AKI is an excellent example of how a fundamental research project with a defined aim, in this case under-

standing acid-base homeostasis, can lead to an unexpected finding that has major implications for human health. While examining proteins expressed by ICs in the context of their proton secretory activity, we were struck by the high expression levels of the P2Y₁₄ protein and set out to examine its role in IC biology. This led us to the discovery that its ligand, UDP-glucose, is a danger-signaling molecule, released by damaged cells, that moves into the kidney and stimulates ICs to produce chemoattractant cytokines, which provoke renal inflammation that is implicated in the onset of AKI. Many known conditions lead to AKI, and individuals who have experienced an episode of AKI in many cases are more prone to develop CKD in the future. We anticipate, therefore, that by understanding the basic cellular mechanisms that are involved in AKI pathogenesis, we can develop new strategies to prevent the development of AKI, which will in turn reduce the incidence of CKD in the population.

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