Conversion of extracellular ATP into adenosine: a master switch in renal health and disease

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Abstract | ATP and its ultimate degradation product adenosine are potent extracellular signalling molecules that elicit a variety of pathophysiological functions in the kidney through the activation of P2 and P1 purinergic receptors, respectively. Extracellular purines can modulate immune responses, balancing inflammatory processes and immunosuppression; indeed, alterations in extracellular nucleotide and adenosine signalling determine outcomes of inflammation and healing processes. The functional activities of ectonucleotidases such as CD39 and CD73, which hydrolyse pro-inflammatory ATP to generate immunosuppressive adenosine, are therefore pivotal in acute inflammation. Protracted inflammation may result in aberrant adenosinergic signalling, which serves to sustain inflammasome activation and worsen fibrotic reactions. Alterations in the expression of ectonucleotidases on various immune cells, such as regulatory T cells and macrophages, as well as components of the renal vasculature, control purinergic receptor-mediated effects on target tissues within the kidney. The role of CD39 as a rheostat that can have an impact on purinergic signalling in both acute and chronic inflammation is increasingly supported by the literature, as detailed in this Review. Better understanding of these purinergic processes and development of novel drugs targeting these pathways could lead to effective therapies for the management of acute and chronic kidney disease.

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ATP maintains energy homeostasis and metabolism[1] and is found at high concentrations (3–5 mM) within the cell. Despite these high intracellular concentrations, extracellular ATP exists at micromolar concentrations under physiological conditions as ATP cannot freely cross the cell membrane owing to its negative charge. However, ATP can be released during apoptosis in a regulated manner through pannexin hemichannels or extruded from necrotic cells[2]. Further, endothelial cells and activated inflammatory cells can release ATP through connexin hemichannels[3].

Release of ATP from the cell allows it to initiate various extracellular purinergic signalling pathways. Purinergic signalling has been shown to regulate physiological processes in the kidney such as water and sodium homeostasis, tubular, glomerular and vascular functions, renal blood flow and autoregulation. Pathophysiologically, the role of purinergic signalling in renal inflammation, hypertension, lithium-induced nephrogenic diabetes insipidus and polycystic kidney disease (PKD) has been well-documented. Purinergic signalling therefore represents an avenue for the development of therapeutic interventions for these diseases. Further, research into purinergic signalling in the immune system, in particular on regulatory T (T_{reg}) cells, is rapidly evolving and has provided insights into the mechanisms of immunological tolerance in renal transplantation.

Here, we review the role of purinergic signalling in the kidney in the context of physiology and pathology. The role of purinergic signalling in kidney health, various models of renal disease and transplantation will be discussed, with an emphasis on ectonucleotidases. We focus on ectonucleoside triphosphate diphosphohydrolase 1 (NTPDase 1; also known as CD39) and ecto-5′-nucleotidase (5′-NT; also known as CD73), which modulate inflammatory states driven by extracellular ATP and anti-inflammatory states driven by extracellular adenosine through the hydrolysis of ATP to adenosine. We finally discuss the lack of clinically available therapeutics targeting purinergic receptors, and how this might be addressed by developing novel drugs targeting CD39 activity or by further investigating currently available agents.

Purinergic receptors

Extracellular ATP and ADP interact with the purinergic P2 receptors to promote inflammation. P2 receptors are expressed in all segments of the nephron, and renal

Key points

- Extracellular ATP and/or ADP acting through P2 receptors exert profound effects on the immune system and also have an impact on kidney pathophysiology.
- Extracellular adenosine is a signalling molecule in the kidney and in the immune system that acts through P1 or adenosine receptors, often opposing the effects of P2 receptor signalling.
- The balance between extracellular ATP and adenosine concentrations is largely regulated by the activity of the CD39–CD73 axis and other ATP hydrolysing enzymes, such as the nucleotide pyrophosphatase/phosphodiesterase proteins.
- CD39 and CD73, an ecto-5′-nucleotidase, constitute a key 'extracellular master switch' in the kidney in both health and disease.
- CD39 plays a role in renal inflammation, immunomodulation, acute kidney injury, chronic kidney disease, diabetic nephropathy, polycystic kidney disease, transplantation and renal cell carcinoma.
- Therapeutic options for kidney disease could include using currently available drugs or developing agents to target purinergic processing.

cells often express multiple receptor subtypes at both the apical and basolateral cell membranes[4,5]. The 15 cell-surface P2 receptors belong to two subclasses: the G protein-coupled P2Y receptors and the ATP-gated P2X non-selective ion channels[6]. The eight P2Y receptors are coupled to different types of G proteins and are activated with differing selectivity by ATP, ADP, UTP and UDP. Given that the focus of this Review is on the conversion of extracellular ATP into adenosine, the activation of P2Y receptors by UTP and UDP is not discussed. The seven P2X receptors are trimeric ligand-gated ion channels that are activated only by ATP. P2X7 is the predominant P2 receptor involved in inflammation and is expressed by virtually all inflammatory cells within the innate and adaptive immune system. Upon activation, P2X7 activates NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasomes and promotes the release of cytokines and chemokines from a range of cells including fibroblasts, mast cells, dendritic cells and peripheral-blood mononuclear cells. P2X7 activation also supports the proliferation and differentiation of T cells to T helper 17 (T_H17) cells and type 1 regulatory T (T_R1) cells and is a potent activator of cell death when expressed on the cell surface (reviewed previously[7]).

Adenosine signals through four G protein-coupled P1 receptors known as the adenosine A_1, A_{2A}, A_{2B} and A_3 receptors. In general, the activation of P1 receptors by adenosine opposes the cellular responses elicited by the nucleotide-mediated stimulation of P2 receptors[8]. Adenosine receptors have traditionally been classified based on their differential coupling to adenylyl cyclase, which acts as a mechanism for regulating cyclic adenosine monophosphate (cAMP) levels. A_1 and A_3 receptors are coupled to the G-inhibitory subunit and their activation leads to a reduction in the level of intracellular cAMP. By contrast, A_{2A} and A_{2B} receptors are coupled to the G-stimulatory subunit and their activation results in an increase in intracellular cAMP. In addition, the A_{2B} receptor couples to Gq proteins, which stimulate phospholipase C activity and intracellular calcium mobilization[9,10]. The A_{2B} receptor has a lower affinity for adenosine than the other P1 receptors, and is principally activated in pathological states when the pericellular concentration of adenosine is high[10]. Using computational modelling, in vitro assays[11] and in vivo studies[12], we have shown that the A_{2B} receptor also interacts with AMP. All four adenosine receptors have been detected on the immortalized human proximal tubular cell line HK-2 (REF.[13]), and although the localization of the adenosine receptors have not been reported in healthy human kidney, the A_{2B} receptor is expressed in the glomeruli and tubules of patients with chronic kidney disease (CKD)[14]. In the rodent kidney, the A_1 receptor is expressed in the glomerulus, proximal tubule cells and afferent arterioles, the A_{2A} receptor in the glomeruli, and the A_{2B} receptor on the vasculature[15]. The A_3 receptor has been identified in whole kidney, but its specific localization is unknown.

Recent evidence has demonstrated that adenosine receptors may form heteroreceptors to modulate functionality; for example, heterodimerization between two adenosine and two dopamine receptors leads to cross talk between these receptors, with implications in the pharmacological management of Parkinson's disease[16]. Within the adenosine receptor family, heterodimerization between A_{2A} and A_{2B} receptors is necessary for A_{2B} receptor to be efficiently expressed on the cell surface in vitro[17]. This may have relevance in the presentation of kidney injury under inflammatory conditions where the A_{2B} receptor is highly expressed[18,19]. The pharmacological, signalling and regulatory properties of adenosine receptors can vary according to cell type within various tissues; although this variation may be due to differing patterns of receptor dimerization between cells, it may also be attributable to receptor modification. Indeed, the properties of G protein-coupled receptors (GPCRs) in general can often differ in a cell-specific and tissue-specific manner attributable to post-translational modifications and/or the association of GPCRs with accessory proteins. However, post-transcriptional mechanisms are also likely to contribute to phenotypic diversity. Although approximately 50% of genes encoding GPCRs are intronless, those that possess introns can undergo alternative splicing, generating GPCR subtype isoforms that may differ in their pharmacological, signalling and regulatory properties[20]. As an example, alternative splicing of the transcript from the gene encoding A_{2A} receptor and differential expression of 5′-UTR variants can decrease receptor protein expression in resting leukocytes or boost the expression of receptors in activated immune cells[20], potentially an important mechanism for how these receptors contribute to the physiological response to sepsis[21]. Consequently, reduced expression of the A_{2A} receptor might be beneficial in the treatment of infection and sepsis[22].

Purinergic regulatory pathways

Intracellular ATP is involved in energy transfer, whereas in the extracellular environment ATP functions as a damage-associated molecular pattern (DAMP) and stimulates an inflammatory response. Although ATP release promotes injury, this process is also essential in facilitating tissue repair[23]. A number of enzymes can hydrolyse extracellular ATP and thus regulate the DAMP activity of ATP by controlling the purinome. The mechanisms by which these enzymes regulate the purinome are described in this section.

G proteins
A family of GTPase proteins involved in transmitting signals from extracellular stimuli to the cell interior.

Damage-associated molecular pattern
(DAMP). Molecules released from damaged or dying cells that trigger immune responses by interacting with pattern recognition receptors.

Purinome
All the proteins in a cell that utilize purine cofactors.

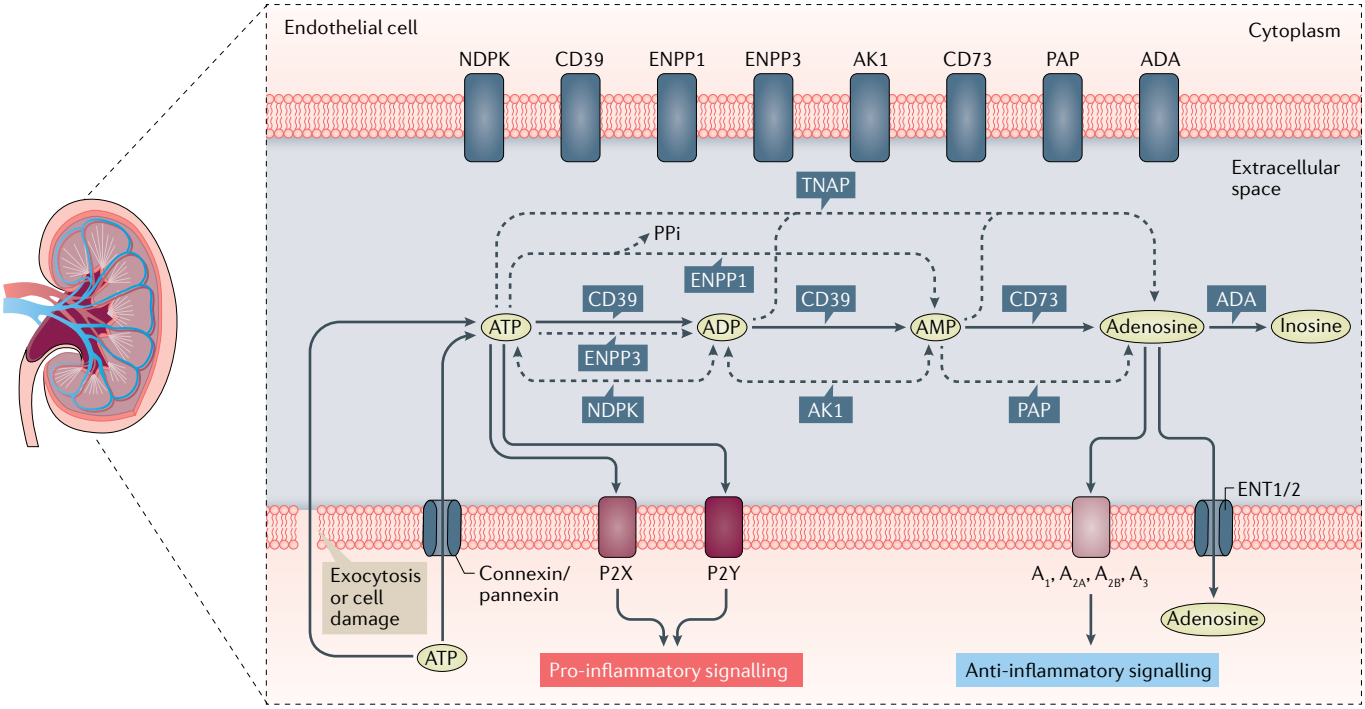

Fig. 1 | **ATP–adenosine metabolic pathways within the kidney.** ATP is released into the extracellular space upon cell damage or through connexin and pannexin hemichannels. Extracellular ATP can trigger pro-inflammatory signalling events by binding the P2X and P2Y receptors. The canonical pathway of ATP metabolism (filled arrows) occurs through the sequential hydrolysis of ATP by CD39 to ADP and AMP, which in turn is hydrolysed to adenosine by CD73. Adenosine may be hydrolysed to inosine via adenosine deaminase (ADA) or taken up by cells through equilibrative nucleoside transporters (ENT1/2). Adenosine also interacts with the adenosine P1 receptors A_1, A_{2A}, A_{2B} and A_3 to mediate anti-inflammatory signalling pathways. Non-canonical metabolic pathways also operate in the extracellular space (dashed arrows). ATP can be metabolized to AMP by ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), releasing pyrophosphate (PPi) or to ADP by ENPP3. Regeneration of ATP can occur through the activity of nucleotide diphosphokinase (NDPK) and adenylate kinase 1 (AK1) through reversible phosphotransfer reactions. Tissue-non-specific alkaline phosphatase (TNAP) can directly convert ATP into adenosine and prostatic acid phosphatase (PAP) may convert AMP into adenosine.

Ectonucleotidases. ATP signalling is tightly regulated by ectonucleotidases. These include ectonucleoside triphosphate diphosphohydrolases (NTPDases) such as CD39, which rapidly hydrolyse ATP and ADP to AMP. The resulting AMP is degraded to adenosine by the 5′-ectonucleotidase CD73, and extracellular adenosine is then converted into inosine by adenosine deaminase.

Four membrane-bound NTPDases have been characterized: CD39 (also known as NTPDase 1), CD39L1 (also known as NTPDase 2), NTPDase 3 and NTPDase 8. Aside from CD39, which hydrolyses ATP and ADP with equal potency, each NTPDase subtype differentially hydrolyses ATP or ADP; CD39L1 preferentially hydrolyses ADP over ATP, and NTPDase 3 and NTPDase 8 preferentially hydrolyse ATP over ADP[24]. CD39 is expressed widely within the immune system and on the vascular endothelium; in the kidney, CD39 is expressed on the vascular endothelium and within the collecting ducts in both the cortex and the medulla. It is also expressed in the ascending thin limb of the loop of Henle[25,26] and the glomerulus[26].

CD73 is predominantly expressed in the glomeruli and on peritubular fibroblasts[27]. Within the kidney, the enzymatic efficiency of CD73 is less than that of CD39 (REF.[28]), resulting in ATP being degraded more rapidly than adenosine is generated. The CD39–adenosine axis is the predominant purinergic pathway in renal pathophysiology and immune regulation; however, there are a number of other enzymes that have an impact on the purinome (FIG. 1).

Ectonucleotide pyrophosphatases/phosphodiesterases. Extracellular ATP can be hydrolysed in one step to AMP and pyrophosphate (PPi) by ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1), or to ADP by ENPP3 (REF.[29]). Both of these enzymes are expressed in the kidney[26,30]. Adenosine can also be generated from nicotinamide adenine dinucleotide (NAD^+) through the coordinated action of ADP-ribosyl cyclase 1 (CD38), ENPP1 and CD73 (REF.[31]). In acute kidney injury (AKI), NAD^+ levels are substantially reduced, having an impact not only on energy homeostasis but also on the regulatory functions of the kidney. Conversely, augmenting NAD^+ and preserving the capacity to generate adenosine protects against ischaemically induced AKI[32]. The activity of these phosphatases have been linked to disease; for example, increased expression of ENPP1 causes insulin resistance and K121Q polymorphisms of

the *ENPP1* gene have been associated with the development of diabetic nephropathy, although the underlying molecular mechanisms have not been elucidated[33]. The same polymorphism was recently associated with acute kidney transplant rejection[34]. PPi, a product of ENPP1 activity, is an inhibitor of hydroxyapatite formation and has been shown to inhibit vascular calcification in rats[35]; vascular calcification is a major complication of end-stage kidney disease, contributing to the excess morbidity and mortality experienced by this patient group[36].

Membrane-bound tissue non-specific alkaline phosphatase (TNAP) expressed in the proximal tubule of the kidney[26] hydrolyses PPi to inorganic phosphate and may play a role[37] in the pathophysiology of vascular calcification and renal stone formation[38]. In a mouse model of CKD, TNAP expression and activity are increased[39] and PPi levels are reduced by dialysis[40,41]. TNAP may also directly hydrolyse ATP, ADP or AMP to adenosine[42], although the physiological significance of TNAP-mediated adenosine production is debated given the high concentration of substrate required. However, in the pathological state of sepsis-associated AKI, exogenous alkaline phosphatase may be a useful therapy because of its dual abilities to both detoxify endotoxin and drive the conversion of ATP into adenosine[43]. Prostatic acid phosphatase also catalyses AMP hydrolysis; however, the distribution and role within the kidney is not well understood[44]. The hydrolysis of ATP and ADP by pyrophosphatases can be counteracted by the activity of membrane-bound and soluble adenylate kinase isoenzyme 1 (AK1) and nucleoside diphosphokinase (NDPK). These enzymes contribute to the regeneration of extracellular ATP by catalysing reversible phosphotransfer reactions[45].

Purinergic signalling in renal tubules

Extracellular ATP released from epithelial cells, acting through P2 receptors expressed in apical and basolateral membranes, regulates renal tubular transport processes. These processes have been extensively reviewed recently[46]; therefore, we limit the discussion here to a few relevant features.

Purinergic signalling mediated by extracellular ATP and other nucleotides opposes the actions of the hormones vasopressin and aldosterone on renal collecting ducts, thus causing diuresis and natriuresis. Purinergic signalling has emerged as one of the major autocrine or paracrine regulators of salt and water handling in the kidney and thus in maintaining water and electrolyte homeostasis. One of the best examples of the purinergic master switch is seen in tubuloglomerular feedback; following increased salt concentration in the distal nephron, ATP is released from macula densa cells. Extracellular ATP is then degraded by CD39, thus releasing adenosine, which triggers constriction of the afferent arteriole and reduces the single-nephron glomerular filtration rate[47,48].

Studies have also revealed interactions between purinergic signalling and the vasopressin V2 receptor–cAMP system, and between purinergic signalling and prostanoid signalling in kidney health and disease[49]. These findings have pathophysiological and therapeutic significance; for example, in a model of lithium-induced nephrogenic diabetes insipidus, genetic deletion of the purinergic receptor P2Y2 protected against polyuria, natriuresis, kaliuresis, collecting duct remodelling and cell proliferation[50,51]. This protective effect is associated with marked changes in the expression and activity of the EP3 prostanoid receptor EP3-I[52]. Furthermore, pharmacological antagonism of the ADP-activated P2Y12 receptor using the anti-thrombotic drugs clopidogrel or prasugrel increased urinary vasopressin excretion in healthy rodents and almost completely ameliorated lithium-induced nephrogenic diabetes insipidus ($P < 0.05$)[51,53,54]. In line with these findings, mice lacking the P2Y12 receptor were found to be significantly resistant to the development of lithium-induced nephrogenic diabetes insipidus[55]. These data suggest a role for ATP/ADP signalling via P2Y receptors in the renal handling of water and in the genesis of nephrogenic diabetes insipidus. Interestingly, blockade of P2Y12 receptor using anti-platelet drugs also increased the levels of circulating vasopressin, apparently through a direct effect on the hypothalamus. In keeping with these observations, mice overexpressing human CD39 (REF.[56]) had impaired urinary concentrating ability[57]. These mice had a decreased urine output and increased urine osmolality in response to water deprivation; however, the mice failed to concentrate urine adequately and had an impaired response to exogenous arginine vasopressin (AVP) when water was restricted. This effect, which occurred with normal vasopressin levels, may be mediated by signalling through the adenosine A_{2B} receptor. Alternatively, enhanced nucleotide scavenging caused by higher levels of CD39 might modify the release of endogenous vasopressin in response to urine volume depletion. Indeed, the overexpression of human CD39 impaired late natriuresis[58] under conditions of high sodium load with concomitant infusion of aldosterone.

Interesting observations were also made with respect to ATP in the tubular lumen. In general, luminal nucleotides are tonic inhibitors of tubular transport[59,60]. However, primary cilia sense urine flow, which modulates tubular transport processes by triggering ATP release and purinergic signalling[61,62]. More details of the role of purinergic signalling in renal tubular transport functions and pathophysiological processes can be found in recent excellent reviews[63–65].

Inflammation and purinergic signalling

Inflammasomes are intracellular cytosolic multi-proteins activated in response to a variety of signals including infection, tissue damage and metabolic dysregulation. Inflammasomes mediate the production of the pro-inflammatory cytokines IL-1β and IL-18, as well as a form of pro-inflammatory cell death known as pyroptosis[66]. The NLRP3 inflammasome is the best characterized and has been implicated in the pathogenesis of a number of renal conditions, including AKI, CKD, diabetic nephropathy and crystal-related nephropathy[67].

Recent studies have highlighted the role of purinergic signalling in the activation of the NLRP3 inflammasome. For example, in response to nanoparticles such as uric acid, silica or alum, renal macrophages release ATP via exocytosis or through connexin and/or pannexin

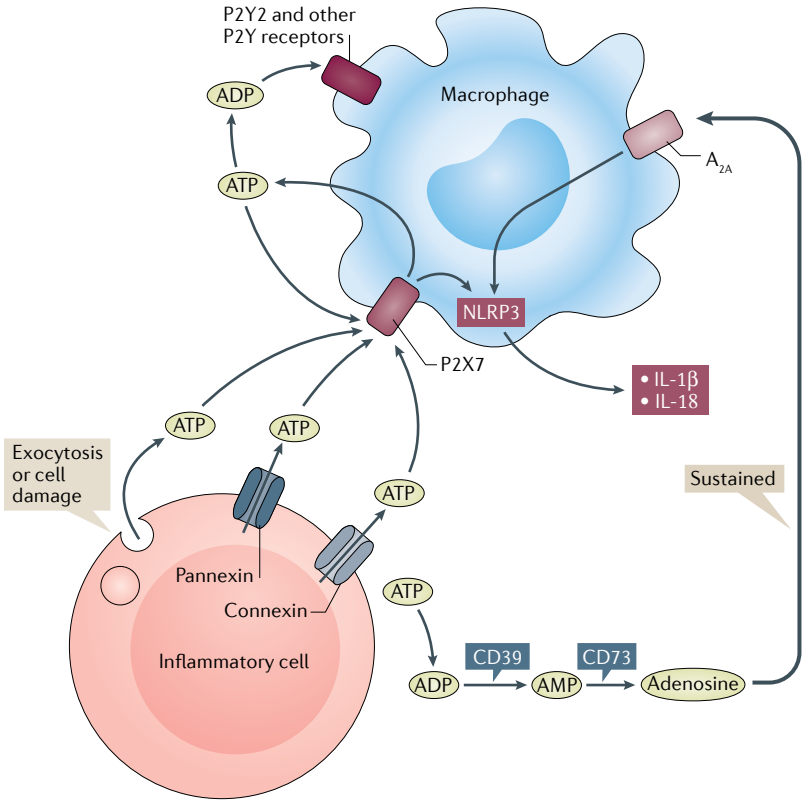

Fig. 2 | **CD39, ATP and adenosine in the activation of the NLRP3 inflammasome.** During inflammation, cells release ATP through exocytosis and pannexin and connexin channels. ATP and ADP are important signalling molecules that allow activation of the NLRP3 inflammasome and the secretion of mature IL-1β and IL-18. Extracellular ATP acts through the P2X7 receptor, amplifying ATP release and increasing the pericellular ATP concentration. ADP stimulates IL-1β production through P2Y receptors, including P2Y2. Sustained increases in pericellular adenosine promote IL-1β secretion through A_{2A} receptor signalling.

hemichannels; ATP can then activate extracellular P2X7 receptors in an autocrine manner and subsequently activate the NLRP3 inflammasome, resulting in the secretion of IL-1β[68–70]. The NLRP3 inflammasome can also be activated by the P2X7 receptor in a paracrine manner, in response to ATP released following hypoxic injury. Activation of P2X7 receptors may further amplify ATP release from macrophages[71,72]. Increased ATP levels are accompanied by increased expression of P2Y receptor mRNA and activation of PLCβ, which promote NLRP3 inflammasome activation in primed macrophages[68,71]. These data show that multiple purinergic signalling pathways are involved in the activation of the NLRP3 inflammasome by ATP. In addition, ADP may stimulate IL-1β production through P2Y receptors expressed on macrophages[71]. Inflammation mediated by the NLRP3 inflammasome significantly contributes to the progression of CKD and is a potential therapeutic target[73–75].

Phagocytosis of necrotic cells promotes potassium leakage from the cell that, combined with increased extracellular ATP concentrations and the activation of P2X7 receptors, is essential for NLRP3 inflammasome activation in macrophages[76]. Manipulation of any of these factors, such as removal of extracellular ATP through the incubation of cells in vitro with apyrase, a soluble form of CD39, inhibits IL-1β secretion[77]. Furthermore, mice with hemizygous knockout of *Entpd1* (the gene that encodes CD39) experienced increased activation of the NLRP3 inflammasome, augmented IL-1β release and venous thrombosis[78].

Intriguingly, adenosine generated after nanoparticle-induced ATP release promotes inflammation and NLRP3 inflammasome activation through A_{2A} and A_{2B} receptors on lipopolysaccharide-primed bone-marrow-derived macrophages[68]. Furthermore, adenosine production is a key regulator of inflammasome activity, increasing the duration and amplitude of the response through activation of the A_{2A} receptor and downstream HIF1α signalling[79]. Indeed, during and after inflammasome activation, prolonged elevated pericellular adenosine concentrations can augment inflammasome activation[79]. It is interesting that administration of apyrase, leading to transient elevated adenosine concentrations, mitigates IL-1β production in vitro, suggesting a reduction in inflammasome signalling, whereas inhibition of adenosine catabolism or the use of stable adenosine agonists, leading to sustained elevated adenosine concentrations, can augment inflammasome activation (FIG. 2).These data are similar to paradoxical findings in which adenosine signalling through P1 receptors has beneficial roles in acute inflammation, whereas sustained elevated adenosine levels aid in the progression to CKD by promoting kidney fibrosis[18].

Ectonucleotidases and immunity

The kidney is often the target of autoimmune diseases due to the presence of many potential renal auto-antigens, and also shows indirect manifestations of systemic autoimmune disorders[80,81]. Purinergic signalling is intricately involved in inflammation and CD39, which is expressed widely on circulating immune cells, was first recognized as a marker of B cell activation[82]. CD39 has now been described on a variety of immune cells across different tissues and organisms, such as: $CD4^+$ T cells (including T_{reg} cells in mice[83] and in humans[84]), $CD8^+$ T cells, neutrophils, macrophages, natural killer cells, natural killer T (NKT) cells, dendritic cells[85] and Langerhans cells in the skin[86]. Other nucleotidases can also influence immune cell function. ENPP1 plays a regulatory function in immune cells such as neutrophils, macrophages, dendritic cells, natural killer cells, and B lymphocytes[87] and has emerged as a therapeutic target for some metabolic diseases[88]. ENPP2, also known as autotaxin, is specifically upregulated on pulmonary NKT cells following hyperoxia and exacerbates lung injury[89]. ENPP2 also possesses lysophospholipase D activity, and the end product of this activity, lysophosphatidic acid, is a bioactive phospholipid involved in various cellular functions, including migration, proliferation and survival (reviewed previously[90]). In newborns, there is an abundance of TNAP expressed on the surface of circulating neutrophils compared with adults[91]. Much less is known regarding the expression of ENPP proteins or TNAP by immune cells and their relevance to renal disease in adults. The role of ectonucleotidase expression is discussed below in the context of inflammation and renal disease.

Regulatory T cells. T_{reg} cells are involved in the pathophysiology of kidney disease and transplantation. On murine T_{reg} cells, CD39 works in tandem with CD73 to produce adenosine; adenosine then acts directly on T cells to suppress their activation through signalling cascades controlled by the A_{2A} receptor[83]. Indeed, T_{reg} cells from mice lacking *Entpd1* experience allograft skin rejection[83] and colitis[92]. Human T_{reg} cells do not express CD73, implicating a paracrine mechanism for the generation of adenosine whereby CD73 is expressed on target cells[84]. Human memory T_{reg} (mT_{reg}) cells, the most stable and suppressive subset of the $CD4^+$ T_{reg} cells, express CD39 and produce adenosine[84,93]. CD39 expression on T_{reg} cells is increased by a positive feedback loop, in which the *Entpd1* promoter is transactivated by adenosine[94]. CD39 expression also decreases T_{reg} cell susceptibility to ATP-induced cell death[95].

T_R1 cells are a subset of T_{reg} cells characterized by the expression of CD39, the secretion of IL-10 and the lack of forkhead box protein P3 (FOXP3) expression. T_R1 cell differentiation is initially promoted by hypoxia through the transcription factors hypoxia-inducible factor 1α (HIF1α) and aryl hydrocarbon receptor. Interestingly, HIF1α and aryl hydrocarbon receptor both compete for the aryl hydrocarbon receptor nuclear translocator ARNT, otherwise known as HIF1β, and protracted hypoxia might paradoxically limit transcription of *Entpd1* in chronic inflammatory states. CD39 contributes to the suppressive activity of T_R1 cells in inflammation by generating adenosine in conjunction with CD73 expressed on the surface of responder T cells and antigen-presenting cells[96]. The pathogenic role of T_R1 cells has been demonstrated in a number of models of inflammation, including autoimmune diabetes, stem cell transplantation and multiple sclerosis[97].

T helper cells. Purinergic signalling influences responses downstream of cytokine signalling. T cells from *Entpd1*-null mice are biased towards T_H1 cell differentiation, which is associated with increased IFNγ production and the spontaneous development of autoimmune alopecia[98]. ATP promotes a pathogenic T_H17 cell phenotype[99], whereas a separate subset of suppressive T_H17 cells have high levels of CD39, co-express CD73 and generate adenosine. These cells have a phenotype similar to T_{reg} cells and the levels of these cells are reduced in people with inflammatory bowel disease[100]. Our own studies, along with others, indicate that purinergic signalling can influence T cell effector responses and chronic inflammatory disease. These responses are reviewed in detail elsewhere[101].

CD39 is upregulated on dendritic cells in response to IL-27 and mitigates inflammation in the central nervous system by limiting the generation of T_H1 and T_H17 cells[102]. In a mouse model of experimental autoimmune encephalomyelitis, IL-27-mediated CD39 upregulation lowered the extracellular concentration of ATP and downregulated nucleotide-dependent activation of the NLRP3 inflammasome[102]. The putative roles of CD39 and adenosine signalling in kidney tissue injury and repair are shown in FIG. 3.

Monocytes and macrophages. Macrophages are monocyte-derived tissue effector cells, present in large numbers in many forms of acute and chronic kidney disease. Macrophages have both pro- and anti-inflammatory roles in the kidney[103,104] and macrophage polarization plays a critical role in the progression of a number of kidney diseases, such as obstructive uropathy, ischaemia–reperfusion injury (IRI), glomerulonephritis, and diabetic nephropathy, among others[105]. Traditionally, macrophages have been classified as either 'classically activated' (M1) or 'alternatively activated' (M2), although it is likely that these states represent extremes of a continuum of activation states. The inflammasome is important in the polarization of macrophages. In M1 macrophages the expression of NLRP3 is high, whereas it is reduced in M2 macrophages[106]. In macrophages with an intermediate activation status (not phenotypically M1 or M2), inflammasome activation is altered and activating molecules, such as extracellular ATP, can become inflammasome inhibitors[107]. The M1 and M2 macrophage phenotypes mirror the polarization of T cells into T_H1 and T_H2 cells[108,109]. M1-polarized macrophages are characterized by the expression of high levels of pro-inflammatory cytokines, whereas M2-polarized macrophages have immunoregulatory functions and promote tissue repair[110]. ATP promotes M1 polarization through P2X7 receptor signalling[111], which occurs early after renal injury and propagates inflammation, and adenosine promotes M2 polarization and tissue repair[112]. Polarization in turn affects ectonucleotidase expression; CD39, together with TNAP, is highly-expressed on M1 macrophages[111] and levels decrease on M2 polarization[106]. By contrast, CD73 expression is strongly downregulated in M1 macrophages, with expression levels recovering on M2 polarization. The differential expression of CD39 and CD73 suggests that adenosine production forms a regulatory 'brake' on macrophage-mediated inflammation[113]. ENPP protein activity has not been demonstrated on M1 macrophages, but *ENPP1* transcription is upregulated on M2 polarization[106].

Vascular endothelial cells. CD39 is the major NTPDase expressed on endothelial cells and associated vascular smooth muscle and plays a key role in mitigating thrombotic and inflammatory events[114]. CD39 is mainly expressed on the endovascular luminal surface in the presence of stable blood flow and is vulnerable to disturbed blood flow[115]. There is evidence that unidirectional laminar sheer stress is associated with an increase in CD39 expression in human endothelial cells, protecting against progressive atherosclerotic disease[115]. CD39, CD39L1, NTPDase 3 and NTPDase 8 are also found in the circulation bound to microparticles[116]. Together with soluble AK1 (REFS[116,117]), these circulating ectonucleotidases constitute an important effector system during inflammatory states. CD73 is also crucial for maintaining endothelial barrier function[118] by generating adenosine, which in turn activates A_{2B} receptors[119].

Platelets. Inflammation is closely linked to immune processes and thrombosis, and platelets are critical mediators in this process. Platelets release ADP from intracellular

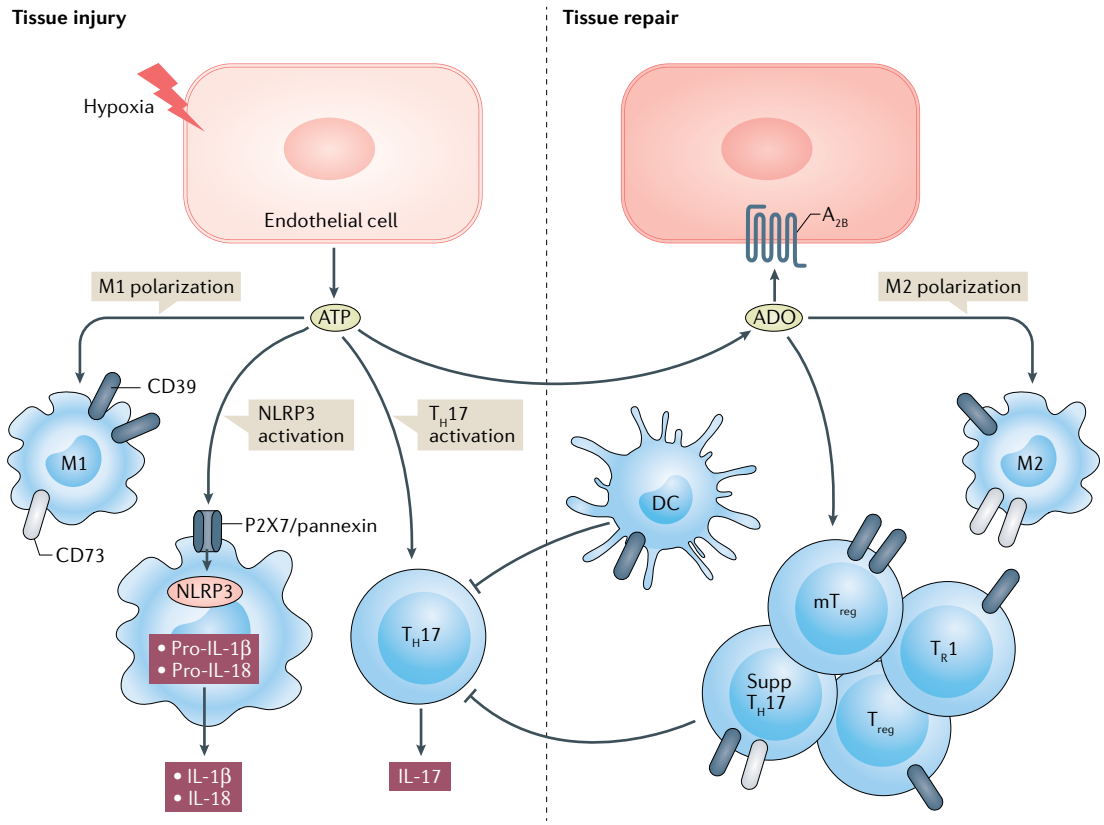

Fig. 3 | **CD39 and regulatory cell purinergic signalling modulates tissue injury and repair.** ATP is extruded from hypoxic cells and promotes tissue injury through activation of the inflammasome and production of IL-1β and IL-18, M1 polarization of macrophages and activation of $CD4^+$ cells such as T helper 17 (T_H17) cells to produce IL-17. The sequential hydrolysis of ATP to adenosine via CD39 and CD73 promotes the resolution of tissue injury through adenosine signalling via the A_{2B} receptor on endothelial cells, or through the promotion of macrophage M2 differentiation and the recruitment of regulatory T (T_{reg}) cells by adenosine. T_{reg} cells, memory regulatory T (mT_{reg}) cells, type 1 regulatory T (T_R1) cells and suppressive (supp) T_H17 cells all express CD39. Under conditions of increased IL-27, dendritic cells (DCs) increase CD39 expression and inhibit T_H1 and T_H17 pathogenic cells. CD39 is the predominant ectonucleoside triphosphate diphosphohydrolase (NTPDase) expressed on M1 macrophages that, together with CD73, enables hydrolysis of ATP to adenosine, forming a regulatory 'brake' on macrophage-mediated inflammation. ADO, adenosine.

granules when activated; this molecule, together with ATP, is intimately involved in the process of thrombosis and haemostasis through the activity of purinergic G protein-coupled receptors. ADP interacts with the P2Y1 and P2Y12 receptors, and ATP acts through P2X1 receptors[120]. Antagonists of the P2Y12 receptor such as clopidogrel, ticlopidine and ticagrelor are used clinically in the management of arterial thrombosis. The diadenosine tetraphosphate derivative GLS-409 targets both P2Y12 and P2Y1 receptors and immediately attenuates platelet-mediated thrombosis[121], but is not currently in clinical use. The P2X7 receptor also plays a role in coagulation as P2X7 receptor activation drives the release of tissue factor, triggering thrombosis[122]. Recent studies conducted in our laboratory indicate that the P2Y12 receptor is expressed in the kidney, especially in the proximal tubular brush border, in the apical domain of collecting duct cells and in arterioles, and anti-thrombotic drugs such as clopidogrel and prasugrel have a significant effect on urinary concentration in health and disease[53,54,58]. Endothelial cell expression of CD39 hydrolyses extracellular ATP and ADP, thereby regulating platelet activation, and adenosine itself blocks platelet activation through the A_{2A} receptor[123].

Purinergic signalling and kidney disease

As purinergic signalling and the activity of ectonucleotidases regulate kidney and immune system homeostasis, it is perhaps unsurprising that deregulation of purinergic signalling has been linked to inflammatory and auto-immune renal diseases. Below, we review the involvement of purinergic signalling in renal disease, with a focus on ectonucleotidase activity.

Renal ischaemia–reperfusion injury. AKI induced by IRI is common in the clinical setting. The influence of CD39 on this type of kidney injury has been comprehensively reviewed[124,125]; therefore, we review here recent advances regarding CD39 and purinergic signalling in renal ischaemia. There is limited evidence supporting the role of other ectonucleotidases, such as the ENPPs and TNAP, in renal IRI.

Kidney injury causes an increase in extracellular ATP concentration and concurrent loss of ectonucleotidase

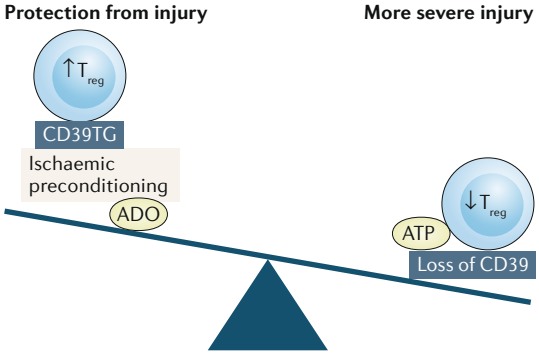

Fig. 4 | **Proposed effects of CD39 and adenosine in acute ischaemic kidney disease.** In ischaemia, ATP is released from cells and promotes kidney injury, whereas adenosine signalling protects against ischaemic-induced renal injury. CD39 modulates the pericellular concentrations of ATP and adenosine (ADO); deficiency in CD39 is associated with severe ischaemic renal injury, whereas the overexpression of CD39 (CD39TG) potently protects against it. Ischaemic preconditioning increases adenosine levels in the kidney and increases the number of regulatory T (T_{reg}) cells, minimizing further injury. T_{reg} cells are critical for promoting tissue repair and depletion of T_{reg} cells exacerbates renal injury.

expression from proximal tubules, resulting in the sustained activation of P2 receptors[65]. Global knockout of *Entpd1*, the gene encoding CD39, resulted in more severe renal injury than mice expressing wild-type *Entpd1* in a mouse model of renal IRI[126,127], whereas mice overexpressing human CD39 were robustly protected[128], highlighting the importance of this ectoenzyme in moderating kidney injury. Expression of human CD39 restricted to either circulating cells or renal tissue protected against injury; this protection could be abrogated with agonists against A_{2A} receptor[128]. Similarly, exogenously administered apyrase reduced renal injury through the catabolism of ATP[18].

Ischaemic preconditioning can mitigate renal injury from subsequent prolonged ischaemia by upregulating CD39 on endothelial cells through the activity of the transcription factor specificity protein 1 (SP1)[129], the generation of adenosine[126] and signalling through A_{2B} receptors[130]. Conversely, mice deficient in CD39 are resistant to this intervention[126]. These data highlight the importance of CD39 in regulating pathophysiological processes within the kidney through the fine-tuning of the effects of ATP and adenosine.

CD73 is also upregulated rapidly by renal ischaemic preconditioning[131]. Mice deficient in CD73 remain susceptible to renal ischaemia despite ischaemic preconditioning, with protection restored on reconstitution with soluble 5′-ectonucleotidase[131]. The effect of CD73 deficiency in renal IRI is controversial. Some studies reported severe kidney injury in CD73-deficient mice[131,132]; however, our data suggest that CD73 deficiency is protective in a less severe model of renal IRI[12]. Further *CD73*-knockout mice reconstituted with soluble CD73 lost their protection, and wild-type mice pre-treated with CD73 inhibitors were protected from IRI.

T_{reg} cells infiltrate kidneys with IRI and are central to promoting recovery and repair (reviewed previously[133,134]). Depletion of T_{reg} cells prior to ischaemia–reperfusion can augment the infiltration of inflammatory cells into the kidney and exacerbate injury[135]. By contrast, ischaemic preconditioning increases the number of T_{reg} cells[136] and the concentration of adenosine[137] within the kidney, which protects against IRI through enhanced A_{2A} receptor signalling and increasing the cell surface expression of programmed cell death 1 (PD-1)[138]. In people with juvenile idiopathic arthritis, the expression of CD39 on T_{reg} cells is genetically determined; individuals that overexpress CD39, for example, those with the GG genotype (rs10748643), have an enhanced capacity for suppressing inflammatory cytokine production[139]. Whether individuals with this genotype are more resistant to renal IRI is not currently known. The role of CD39 and other factors in influencing IRI is summarized in FIG. 4.

Acute kidney injury and chronic kidney disease. Although historically considered distinct entities, a number of epidemiological and mechanistic studies have demonstrated that AKI and CKD are inter-related. AKI, even in the context of full biochemical recovery, is a risk factor for CKD, end-stage kidney disease, cardiovascular complications and death[140]. The impact of CKD is felt globally and confers a notable economic and health burden[141].

CKD is characterized by the replacement of renal parenchyma with regions of tubulointerstitial fibrosis, leading to the irreversible loss of renal architecture. Tubulointerstitial fibrosis correlates with renal dysfunction clinically — in end-stage renal disease, much of the kidney is replaced with fibrotic tissue[142]. The key cells in the fibrotic process are fibroblasts, which express CD73 and the A_{2B} receptor[143]. Chemical activation of the A_{2B} receptor resulted in increased transcription of profibrotic and inflammatory mediators, including α-actin 2, IL-6, TGFβ, connective tissue growth factor and fibronectin[144]. Fibrosis results in capillary rarefaction and hypoxia, which may further potentiate inflammation and fibrosis[145–147].

Although adenosine signalling limits inflammation acutely, sustained adenosine receptor activation has the opposite effect. Both overexpression of human CD39 and treatment with apyrase robustly protect against renal injury 24 h following ischaemia in a mouse model of acute IRI[126–128]. However, in chronic IRI, these strategies had opposing effects: apyrase reduced renal fibrosis following injury, whereas the overexpression of human CD39 promoted renal fibrosis[18]. The exaggerated fibrotic response in kidneys from mice overexpressing human CD39 was associated with increased pericellular adenosine levels and expression of *Adora2b* mRNA (corresponding to adenosine receptor A_{2B})[148]. The importance of adenosine signalling in the development of renal fibrosis was also highlighted in a study in which mice deficient in adenosine deaminase accumulated adenosine and developed CKD[143]. Further, subcutaneous infusion of angiotensin II increases renal adenosine levels, upregulates A_{2B} receptor expression and results in

renal fibrosis[143] through the upregulation of HIF1α and endothelin 1 (REF.[14]). Finally, humans with hypertensive nephrosclerosis and CKD have high levels of *Nt5e* mRNA and A_{2B} receptor expression[14]. These data suggest that sustained elevated concentrations of adenosine promote fibrosis and may be a consequence of inflammasome activation[68].

It is interesting to note that mice lacking the gene encoding CD73 have an impaired ability to generate adenosine[149] and spontaneously develop renal fibrosis by 6 months of age[150]. Whilst elevated whole-tissue levels of AMP have not been demonstrated in *Nt5e*-knockout mice, they have been shown in wild-type mice following treatment with the CD73 inhibitor α,β-methylene ADP (APCP)[118]. This may be because APCP is a competitive inhibitor of both CD73 and TNAP[27]. In *Nt5e*-knockout mice, extracellular AMP levels may not be elevated owing to the activity of TNAP, an effect previously demonstrated in the human airway[151]. AMP is a ligand for the A_{2B} receptor[11]; therefore, sustained activation of the A_{2B} receptor by AMP, and adenosine generated via TNAP, may promote fibrosis in *Nt5e*-knockout mice in a similar manner to the sustained engagement of the A_{2B} receptor by adenosine.

In a transplant model with a period of extended cold organ preservation[128], mice that received kidneys overexpressing human CD39 had fewer tubular injuries, better renal function and survived for longer than mice that received wild-type kidneys[128]. In this model, transplantation triggers the release of ATP, which, acting as a DAMP, induces sterile inflammation in the organ after implantation and can mediate allograft rejection. Indeed, knockout of *Entpd1* in a mouse model of major histone compatibility (MHC)-mismatched liver transplant resulted in increased inflammation, increased production of IFNγ and enhanced $CD8^+$ T cell infiltration[152]. Furthermore, T_{reg} cell numbers were reduced in CD39-deficient livers.

In a model of unilateral ureteral obstruction (UUO), mice overexpressing human CD39 were susceptible to fibrosis[153]. Expression of *Adora2a* mRNA, but not *Adora2b* mRNA, and endothelin 1 was higher in mice overexpressing human CD39 than wild-type mice 7 days following the initial UUO injury, although adenosine itself was not quantified. As observed in a model of ischemic-induced CKD, sustained adenosine generation and adenosine receptor signalling may be regulated by activation of the NLRP3 inflammasome. In another model of UUO, fibrosis was exacerbated in *Adora2a*-knockout mice, particularly in the initial stages following injury[154]; similarly, A_{2A} receptor activation reduced collagen deposition and the expression of profibrotic markers, and reduced $CD4^+$ T cell infiltration and increased the ratio of T_{reg} to $CD4^+$ T cells[155].

Loss of P2 purinergic receptors both protects against and sensitizes mice to renal injury in models of UUO, depending on the subtype of receptor deleted. Mice lacking the gene encoding the P2X7 receptor (*P2rx7*) were protected from renal fibrosis in the UUO model, despite the fact that the P2X7 receptor is only transiently expressed in tubular epithelial cells[156]. Conversely, *P2rx4*-knockout mice have a heightened susceptibility to fibrosis following UUO compared with wild-type mice[157]. These divergent effects are perhaps not unsurprising given that P2X4 has been shown to interact with P2X7[158] and modulate its pro-apoptotic and pro-inflammatory effects, and its absence may therefore potentiate these effects.

Immune-mediated kidney injury. In an experimental model of antibody-mediated glomerulonephritis in mice, urinary ATP levels were increased in injured mice compared with controls, with a concomitant upregulation of P2Y2 receptor expression in glomeruli and on isolated podocytes and infiltrating leukocytes. Chimeric experiments in which P2Y2 receptor expression was restricted to either the haematopoietic or non-haematopoietic system showed that signalling through the P2Y2 receptor on infiltrating haematopoietic cells played a major role in the development of antibody-mediated glomerulonephritis. CD39 expression throughout the whole kidney, as well as in glomeruli and podocytes, was increased in mice with glomerulonephritis and intra-peritoneal treatment of animals with apyrase reduced urinary ATP levels and decreased the severity of inflammation, as did blocking P2 receptors using the guanine nucleotide-binding protein inhibitor suramin[159]. In a murine model of lupus nephritis, activation of P2X7 signalling and the NLRP3 inflammasome following the induction of disease with Brilliant Blue G in lupus-prone mice resulted in an increase in IL-1β production and enhanced T_H17 cell polarization[160]. Other studies have shown that adenosine signalling via the A_{2A} receptor can attenuate immune-associated inflammatory kidney diseases in rats[161] and mice[148]. These data together highlight that both removal of nucleotides and the generation of adenosine can mitigate inflammation. The direct impact of CD39 activity on the development and progression of glomerulonephritis using genetically modified mice has not been explored.

Renal injury following transplantation. Perhaps the most common form of immune-mediated kidney injury occurs following renal transplantation. Kidney transplantation is the most common transplant procedure and is the optimal form of renal replacement therapy for individuals who reach end-stage renal disease, offering improved quality and length of life compared with dialysis and supportive care[162]. Despite improvements in short-term graft survival with the advent of more potent immunosuppression strategies, long-term graft survival has not markedly changed. The causes of graft loss are complex and multifactorial; however, evidence suggests that the major factor is recurrent acute and chronic antibody-mediated rejection[163]. Lifelong immunosuppression is still a necessity in all but a few patients; immunological tolerance is the ultimate goal of transplantation medicine as it enables long-term allograft survival while avoiding the toxicity associated with immunosuppression.

T_{reg} cell number and function are intimately linked with transplant rejection and tolerance. In humans, CD39 is expressed independently of CD73 by a subset of T_{reg} cells that robustly express FOXP3. Distinct

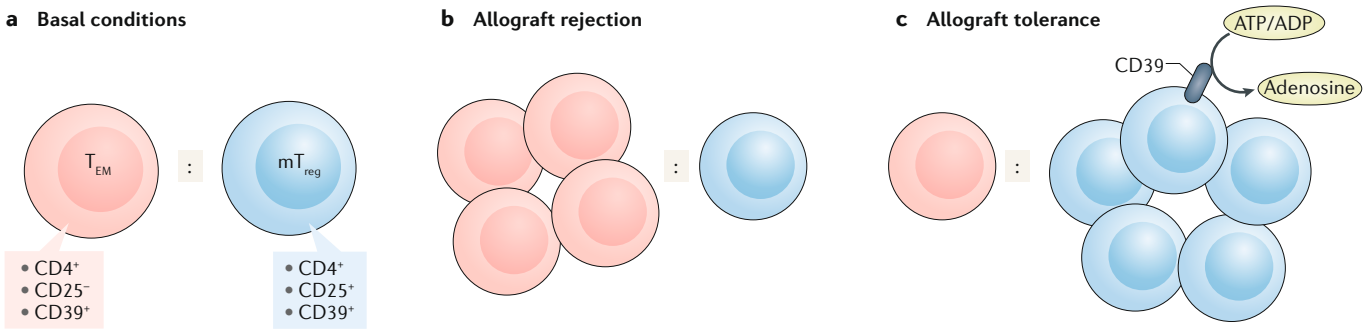

Fig. 5 | **$CD4^+CD25^{+/-}CD39^+$ expression in the peripheral blood of patients with renal allograft rejection in transplantation rejection and states of tolerance.** Under basal conditions, $CD4^+CD25^-CD39^+$ effector memory T (T_{EM}) cells and $CD4^+CD25^+CD39^+$ memory regulatory T (mT_{reg}) cells exist in equal proportions. This ratio does not change markedly in the setting of a stable renal function. In antibody-mediated allograft rejection, the proportion of T_{EM} cells markedly increases compared with mT_{reg} cells. In tolerant transplant recipients, the number of circulating mT_{reg} cells is markedly increased. These cells have greater suppressive function and CD39 expression; thus, the T cell population of patients with stable graft function have an enhanced capacity to degrade extracellular ATP/ADP.

populations of $CD4^+CD39^+$ T lymphocytes can be identified as $CD4^+CD25^+CD39^+$ memory T_{reg} (mT_{reg}) cells and $CD4^+CD25^-CD39^+$ effector memory T (T_{EM}) cells. The ratio of peripheral blood T_{EM} to mT_{reg} cells is increased in patients undergoing renal transplant rejection compared with patients without rejection[84]. mT_{reg} cells in patients with a stable transplant are highly suppressive[164]; this observation is in keeping with previous reports that mT_{reg} cells are the most stable and suppressive subset of $CD4^+$ T_{reg} cells[165]. In tolerant patients with stable kidney graft function who had not taken any immunosuppression medication for at least 1 year, the proportion of circulating mT_{reg} cells was increased compared with patients with stable graft function on immunosuppression medication[166]. Furthermore, mT_{reg} cells from tolerant patients had a statistically significant ($P < 0.05$) greater degree of demethylation in the T_{reg}-specific demethylated region and greater suppressive function than patients with stable graft function[166]. mT_{reg} cells from patients with transplantation tolerance also had greater CD39 expression[166] and enhanced capacity to degrade extracellular ATP/ADP than patients with stable graft function[167] (FIG. 5).

Cell therapy with T_{reg} cells was shown to be protective against adriamycin nephropathy, a model of injury whereby the direct toxicity of adriamycin induces glomerulosclerosis, interstitial fibrosis and proteinuria by 6 weeks following adriamycin administration[168]. Mice that overexpress human CD39 are protected from adriamycin-mediated nephropathy; the transfer of T_{reg} cells from these mice to wild-type mice conferred greater protection from adriamycin-mediated nephropathy than the transfer of wild-type T_{reg} cells, suggesting an additional independent effect of CD39 within the kidney[169]. Notably, T_{EM} cells transferred from mice overexpressing human CD39 to wild-type mice did not confer protection to adriamycin-mediated nephropathy[169], suggesting that T_{EM} cells from these mice do not possess suppressive powers, despite CD39 expression.

Ectonucleotidases other than CD39 have been implicated in kidney transplant rejection. The study of a cohort of white kidney transplant recipients from southern Brazil showed an independent association between the *ENPP1* 121Q/Q genotype, which results in decreased ENPP1 activity, and acute kidney transplant rejection[34]. The authors speculate that the presence of the *ENPP1* Q/Q genotype has an indirect negative effect on adenosine-generating activities during inflammation owing to a reduction in the generation of AMP substrate. Loss of adenosine production is thought to then increase the activity of T effector lymphocytes, typical in acute transplant rejection.

Purinergic signalling in immunity and kidney cancers. Recognition of the role of the immune system within the tumour microenvironment has resulted in a paradigm shift towards the treatment of malignancies with immunotherapy. Whereas augmentation of purinergic signalling is advantageous in the immune-mediated kidney diseases described above, in the setting of solid organ cancers, purinergic signalling suppresses immune responses and promotes angiogenesis and tumour spread[170]. Indeed, accumulation of adenosine within the tumour microenvironment, a result of intra-tumour hypoxia, inhibits the antitumour function of immune cells through interaction with the A_{2A} receptor on these cells[170,171]. Renal cell carcinoma (RCC) may be particularly susceptible to the effects of purinergic signalling, as genes encoding the CD73 and A_{2A} receptors are highly expressed in this cancer[172]. Furthermore, the concentration of T_{reg} cells is higher in the peripheral blood of individuals with RCC than in healthy individuals and is predictive of shortened survival (reviewed previously[173]). A single study showed that in patients with RCC, the presence of ≥10% tumour-infiltrating T_R1 cells ($CD39^+$ $FOXP3^-$) correlated negatively with survival[174]. CD73-deficient mice are resistant to cancer development[175] and anti-CD73 monoclonal antibodies (mAbs) reduce breast cancer growth in mice[176] by reducing the generation of adenosine within the tumour.

The importance of purinergic signalling in tumour development extends beyond adenosine generation; in solid tumours in mice, ATP is released in abundance into the extracellular space. Inhibition of ATP catabolism

T_{reg}-specific demethylated region
An evolutionarily conserved non-coding element within the *FOXP3* gene locus that controls *FOXP3* expression.

using anti-CD39 mAbs resulted in the engagement of P2X7 receptors, inflammasome activation and IL-18 release, with the net effect of enhanced T cell proliferation and a reduction in intra-tumour macrophage function[177]. Recent data demonstrated that anti-CD39 and anti-CD73 mAbs act synergistically to enhance antitumour immunity[178] and could be an effective combination therapy.

Ectonucleotidases other than CD39 and CD73 have also been linked to cancer phenotypes. ENPP1 is overexpressed in human primary breast cancer cells relative to normal mammary epithelium and in bone metastases. Further, in a mouse xenograft model, ENPP1 promoted the development of bone metastasis[179] and resistance to conventional chemotherapy in breast cancer[180]. Similar oncogenic potential for ENPP1 has been demonstrated in lung cancer[181]. Increased ENPP2 expression has been reported in a number of cancers, such as glioblastomas, hepatocellular and thyroid carcinomas, breast, pancreatic and haematological cancers[90] and correlates with metastatic potential. Further, in RCC, ENPP2 activity[182] has been suggested to mediate resistance against the cancer therapeutic sunitinib, a receptor tyrosine kinase inhibitor.

The complexity of purinergic signalling mandates careful evaluation of the entire purinome in emerging cancer therapies. We refer the reader to a recent, comprehensive review of the role of purinergic signalling in cancer[45].

Diabetic nephropathy. Diabetic nephropathy is the leading cause of end-stage renal failure in the western world[183] and is increasing in prevalence in Asia[184]. Kidney biopsies from patients with diabetic nephropathy show progressive renal fibrosis and glomerulosclerosis[185]. Purinergic signalling has been implicated in the pathogenesis of this disease: glomerular hyperfiltration, an early event associated with the onset of diabetic nephropathy, is reduced by A_{2A} receptor activation[186]. In a rat model of diabetic nephropathy, the rate of nucleotide hydrolysis was found to be increased compared with the rate in wild-type rats[187,188]. In addition, individuals with diabetic nephropathy have higher levels of plasma adenosine than patients with diabetes without renal disease[189]. These data suggest that ATP and the scavenging capacity of CD39 may drive glomerular inflammation. Indeed, in a model of diabetes-related renal disease in mice, mice deficient in CD39 exhibit insulin resistance and develop more severe diabetes-related renal disease than wild-type mice. Disease may be driven through the ATP-mediated production of CC-chemokine ligand 2 (CCL2, also known as MCP1)[190] as kidneys from *Entpd1*-null mice with diabetes-related renal disease show more advanced glomerulosclerosis, tubulointerstitial fibrosis, increased glomerular inflammation and increased expression of CCL2. In vitro, *Ccl2* mRNA expression was upregulated in mesangial cells by ATP and UTP, but not by ADP or adenosine[191]. By contrast, mice deficient in the P2X7 receptor were protected from diabetes-induced kidney damage and showed fewer macrophages within the glomeruli, with no increase in collagen IV expression compared with wild-type mice[192].

In humans, two common *ENTPD1* polymorphisms (rs12763743 and rs3897983) show a strong association with the presence of diabetic nephropathy in African Americans with type 2 diabetes mellitus[193]. Further, P2X7 receptor expression was shown to be increased in kidney biopsies from patients with established diabetic nephropathy, along with severe mesangial expansion, interstitial fibrosis and impaired renal function[192]. Together these data implicate elevated ATP concentrations and signalling through the P2X7 receptor in the pathogenesis of diabetic nephropathy. It is therefore likely that other ATP hydrolysing enzymes are involved in diabetic nephropathy, although data on this are somewhat limited. However, the K121Q polymorphism of the *ENPP1* gene is associated with the development of diabetic nephropathy and renal dysfunction[33].

Polycystic kidney disease. Autosomal-dominant polycystic kidney disease (ADPKD) is the most common inherited nephropathy and the fourth most common cause of kidney failure in the Western world[194]. Epithelial cells from individuals with ADPKD were shown to release higher amounts of ATP (from 0.5 mM to 2 mM) than those from healthy controls (350 nM to 500 nM), contributing to high concentrations of ATP (up to 10 mM) within cyst fluid[195]. In keeping with the finding of high ATP concentrations in the cyst fluid of patients with ADPKD, expression levels of P2X7, P2Y2 and P2Y6 receptors on the cystic epithelia were increased and contributed to cyst growth in a rat model of polycystic disease[196]. Another study showed that CD39 expression is reduced in patients with ADPKD in parallel with increased ATP levels[197]. Although intraluminal adenosine was not measured in this study, the authors speculated that adenosine signalling may promote disease progression[198]. Indeed, knockdown of the P2X7 receptor reduced cyst formation in a zebrafish model of ADPKD, suggesting that P2X7 antagonists may have therapeutic potential for this disease[199].

Autosomal-recessive polycystic kidney disease (ARPKD) is a rare form of polycystic disease diagnosed in infants, either before birth or in babies or young children. For children with ARPKD that survive the newborn period, approximately half will reach end-stage kidney disease by 10 years of age. In contrast to the mouse models of ADPKD, in a mouse model of ARPKD, P2X7 receptor agonism reduced cyst formation[200]. Interestingly, P2X7 protein expression was upregulated in human foetal ARPKD epithelia versus normal foetal collecting ducts[200], which, combined with the above animal data, suggests that ATP and P2X7 receptor may be treatment targets for this condition. In another model of ARPKD, ATP levels were increased and their effects on cyst development and progression were mediated through P2X4 and/or P2X7 receptors[201]. For an in-depth review on purinergic signalling in PKD, we refer readers to a recent review[202].

Experimental therapeutics

Exogenous adenosine has been considered as a therapeutic intervention for a number of disease processes; however, the widespread distribution of adenosine

receptors and, in particular, the effect of exogenous adenosine on the heart, has limited the application of adenosine analogues therapeutically. The administration of adenosine clinically is currently limited to the treatment of supraventricular tachycardia; it induces a transient atrioventricular nodal block and restores sinus rhythm. Other therapies that modulate the activity of adenosine receptors such as ectonucleotidases may hold promise as treatments for diseases mediated by purinergic signalling.

Adenosine analogues. Selective adenosine receptor agonists and antagonists are currently available and one A_{2A} receptor agonist — regadenoson — is approved by the FDA and limited to myocardial perfusion imaging in patients with suspected coronary artery disease[203]. Although clearance of regadenoson is dependent on renal excretion, it was tolerated by patients with advanced stage CKD[204,205]. Notably, the A_{2B} receptor is the least well described adenosine receptor, despite being operational in many pathological states, and currently only partial agonists exist for the A_{2B} receptor[206].

The recognition of ATP and adenosine as key players in the host–tumour interaction has recently translated into clinical practice. A recent first-in-human study established that treatment of refractory RCC with an A_{2A} receptor antagonist was safe and efficacious in patients with an adenosine-regulated gene-expression signature in pre-treatment tumour biopsies[172,207]. Furthermore, the importance of ATP in cancer biology has led to the first-in-human trials of an anti-CD39 antibody in patients with advanced cancer (NCT03884556)[177].

P2 receptor ligands. In other distinct renal disorders, targeted P2Y2 receptor antagonism has potential for use in the treatment of acquired nephrogenic diabetes insipidus, such as that induced by chronic lithium therapy (reviewed previously[51]). Conversely, future development of agonists of P2Y2 receptor may be useful in treating conditions such as dilutional hyponatraemia, as experimental evidence has shown that agonistic activation of P2Y2 receptor in the medullary collecting ducts decreases AVP-induced osmotic water reabsorption by reducing the cellular cAMP levels, and *P2ry2*-knockout mice develop higher urinary concentrating ability than wild-type mice owing to sensitization of their kidneys to circulating vasopressin levels[208]. Furthermore, in addition to their anti-thrombotic activity, P2Y12 receptor antagonists may find potential use in the treatment of lithium-induced nephrogenic diabetes insipidus[50,53–55,57].

Augmentation of CD39 activity. A promising emerging therapeutic strategy for a number of disease processes is augmentation of CD39 activity, which should facilitate both ATP removal and adenosine generation. In vitro, CD39 activity can be increased by common therapeutics: CD39 activity was increased on HUVECs incubated with angiotensin-converting enzyme inhibitor[209]; similarly, treatment of activated endothelial cells with an inhibitor of HMG-CoA reductase restored CD39 expression and the metabolism of ATP[210]. The red wine polyphenols quercetin and resveratrol also restored CD39 activity in activated human umbilical vein endothelial cells[211]. The implications of this pharmaceutical activity in kidney health and disease have not been studied in detail; however, in individuals with rheumatoid arthritis who had low expression of CD39 on T_{reg} cells and were refractory to methotrexate therapy, the combination of a HMG-CoA reductase inhibitor (atorvastatin) and the tumour necrosis factor (TNF) inhibitor etanercept provided an added immunomodulatory benefit through the augmentation of $CD39^+$ T_{reg} cells[212].

Sesamin, a major ligand extracted from sesame seed oil, has anti-hypertensive, hypocholesterolaemic, neuroprotective, anti-fibrotic, anti-oxidative, anti-tumour, and anti-inflammatory actions[213–217]. In a model of kidney IRI, treatment with oral sesamin administered in the 24 h prior to ischaemia reduced injury by inhibiting neutrophilic infiltration and producing the pro-inflammatory cytokines TNF and IL-1β. Whereas ischaemia injury induces a loss of CD39 cell surface expression[218], sesamin was found to promote, and in the setting of ischaemia augment, the expression of *Entpd1*, *Adora2a* and *Adora2b* mRNA and the corresponding CD39, adenosine A_{2A} and A_{2B} receptor proteins, as well as adenosine production[219].

Carbon monoxide (CO) modulates the innate immune response associated with IRI in the kidney and accelerates tissue recovery through increased expression of CD39 and the adenosine A_{2A} and A_{2B} receptors. These changes are associated with a >20-fold increase in expression of the circadian rhythm protein hPER2 and a five-fold increase in serum erythropoietin[220]. Similarly, in cardiac ischaemic injury, the protective role of CD39 mediated through the A_{2B} receptor[221] has been linked with the upregulation of hPER2, which enhances the glycolytic capacity of the ischaemic heart through HIF1 stabilization[222].

Ferulic acid, derived from fruits and vegetables, is a potent anti-oxidant of scavenging free radicals. Administered to mice in the 24 h prior to ischaemia, ferulic acid markedly attenuated kidney damage, reduced the infiltration of neutrophils to the ischaemic site and reduced TNF and IL-1β levels as measured by enzyme-linked immunosorbent assay. Ferulic acid also promoted expression of *Hif1a*, *Entpd1* and *Nt5e* mRNA and the corresponding HIF1α, CD39 and CD73 proteins, as well as adenosine production in whole kidney[223].

In peripheral blood, high-intensity exercise increased $CD4^+CD25^+CD39^+$ mT_{reg} cell numbers in healthy men of high and low physical fitness. At baseline, physically unfit individuals had higher proportions of $CD4^+CD25^-CD39^+$ T_{EM} cells and lower numbers of circulating mT_{reg} cells than physically fit individuals[224]. Although exercise training has been studied in the context of cardiovascular outcomes following transplantation[225], its effect on renal transplant rejection is not known.

It should be noted that interventions based on manipulating CD39 should be used with caution. Although mice that overexpress human CD39 are generally phenotypically normal, they have prolonged bleeding times under anaesthesia[56]. Under basal conditions, these mice largely exhibit normal purinergic homeostasis in keeping

with minimal flux and pre-existing low extracellular ATP and ADP levels[18]. To overcome the observed prolonged bleeding times seen with global over-expression of CD39, our laboratory has designed a P-selectin-targeted CD39 molecule (rsol.CD39-PSGL1) consisting of recombinant soluble CD39 that incorporates 20 residues of the P-selectin binding domain of P-selectin glycoprotein ligand 1 (PSGL1). In a renal model of IRI, kidneys were protected from injury with administration of rsol.CD39-PSGL1 30 min prior to ischaemia without a systemic anti-coagulant effect or notable toxicity[226].

Conclusions

Extracellular ATP and its metabolite, adenosine, are signalling molecules that activate P2 or P1 purinergic receptors, respectively. P2 and P1 receptor activation elicits a variety of biological functions in the kidney that are dysregulated under pathophysiological conditions. Although ATP and adenosine act independently as receptor ligands, they are interrelated as adenosine is a hydrolytic product of ATP. This interrelation is regulated by the activity of ectonucleotidases in the kidney. Of these ectonucleotidases, CD39 and CD73 acting in tandem play the key role in mediating purinergic signalling in the kidney, modulating various physiological functions and interacting with the immune system in relation to renal pathophysiology. The accumulated experimental evidence strongly suggests that CD39 specifically functions as a master-switch and rheostat to modulate and fine-tune responses to extracellular purines through mediating P2 and P1 receptor-mediated pathophysiological effects in the kidney. The availability of animal models with altered expression and function of purinergic receptors or CD39, as well as compounds such as soluble apyrase, have helped to advance understanding of the roles of CD39 in a number of conditions of renal pathophysiology. These studies have also provided deeper insights into how CD39 functions in immune cells as a powerful immunomodulator, potentially altering the course of renal pathophysiology. CD39 is a very promising target for the treatment of a number of renal pathophysiological conditions. Gaining further knowledge of the factors that release CD39 from cells and the signals involved in triggering CD39 release will markedly advance our ability to develop CD39-based therapeutic modalities to treat renal diseases.

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