Activated CD47 regulates multiple vascular and stress responses: implications for acute kidney injury and its management

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Rogers NM, Yao M, Novelli EM, Thomson AW, Roberts DD, Isenberg JS. Activated CD47 regulates multiple vascular and stress responses: implications for acute kidney injury and its management. Am J Physiol Renal Physiol 303: F1117–F1125, 2012. First published August 8, 2012; doi:10.1152/ajprenal.00359.2012.—Ischemia-reperfusion injury (IRI) remains a significant source of early and delayed renal transplant failure. Therapeutic interventions have yet to resolve this ongoing clinical challenge although the reasons for this remain unclear. The cell surface receptor CD47 is widely expressed on vascular cells and in tissues. It has one known soluble ligand, the stress-released matricellular protein thrombospondin-1 (TSP1). The TSP1-CD47 ligand receptor axis controls a number of important cellular processes, inhibiting survival factors such as nitric oxide, cGMP, cAMP, and VEGF, while activating injurious pathways such as production of reactive oxygen species. A role of CD47 in renal IRI was recently revealed by the finding that the TSP1-CD47 axis is induced in renal tubular epithelial cells (RTEC) under hypoxia and following IRI. The absence of CD47 in knockout mice increases survival, mitigates RTEC damage, and prevents subsequent kidney failure. Conversely, therapeutic blockade of TSP1-CD47 signaling provides these same advantages to wild-type animals. Together, these findings suggest an important role for CD47 in renal IRI as a proximate promoter of injury and as a novel therapeutic target.

thrombospondin-1; CD47; kidney injury; nitric oxide; reactive oxygen species; ischemia-reperfusion injury; transplant

ISCHEMIA-REPERFUSION INJURY (IRI) is a leading cause of acute renal injury and a major cause of both acute graft dysfunction and delayed renal transplant failure (8, 29). The role of extracellular matrix and matricellular proteins in this process remains largely unknown. The secreted matricellular protein thrombospondin-1 (TSP1) has been associated with acute renal injury and implicated in several preclinical models of chronic renal disease. TSP1 is the only known soluble high-affinity ligand for the ubiquitous cell receptor CD47 (40), although signal regulatory protein-α (SIRP-α) functions on phagocytes as a counterreceptor to CD47 (94). New findings identify CD47 as a regulator of multiple cell survival and cell death pathways (44, 46, 85), suggesting an important role for the TSP1-CD47 axis in renal injury.

TSP1

TSP1 is the first and most widely studied member of a five-member family of secreted proteins (14). It exists in vivo as a trimer of 150-kDa subunits, which with glycosylation has a mass of ~480 kDa. This large size limits TSP1 diffusion across certain tissue barriers such as the endothelial basement membrane. TSP1 was first identified in platelet α-granules (23, 55), where it can account for >50% of the total protein. Following in utero development (16), TSP1 expression in adult animals is minimal except for the preformed pool stored in platelets and circulating at ~100 pM levels in plasma. However, TSP1 can be induced in tissues in response to injury (2) and chronic disease (32). Recently, we reported increased plasma TSP1 in steady-state sickle cell disease (SCD) patients that correlated significantly with vasculopathic complications including acute chest syndrome (76). These observations are made prescient in light of the known increased rate of chronic kidney disease and kidney failure in SCD patients (1). As a secreted protein, TSP1 has no direct intracellular targets but alters cellular responses by interacting with cell surface receptors including several integrins (13), CD36, CD47, hepatic sulfate proteoglycans (15), and LDL receptor-related protein-1/calreticulin (79). Central physiological activities of TSP1 include inhibition of angiogenesis through interaction with the vascular cell receptors CD36 (20) and CD47 (47), binding to
and regulation of extracellular matrix formation and structure, activation of latent TGF-β1 (73), modulation of inflammatory cell adhesion and migration (74), and suppression of tumor growth (84).

CD47

Integrin-associated protein, or CD47, was first identified as a missing antigen in Rh-negative red blood cells (72) and as an overexpressed protein in ovarian carcinoma (11). This membrane-spanning protein also coassociates with SIRP-α and is a receptor for TSP1 (4). TSP1, binding in a high-affinity manner through its C-terminal domain (40), activates CD47 to alter numerous cellular processes. CD47 regulates inflammation, cell adhesion, and self-recognition (9). More recently, CD47 has been found to limit cell and animal survival in response to various stressors, in part through regulation of cardiovascular signaling mechanisms (5, 47, 49).

Distribution of TSP1 and CD47 in the Kidney

TSP1 is expressed in the kidney during development (39), whereas in healthy adults, TSP1 is virtually undetectable. In cell culture or with injury, TSP1 is secreted by kidney mesangial cells, a process that can be suppressed by nitric oxide (NO) pro-drugs (96). CD47 is expressed in several renal cell types including epithelial, endothelial, and mesangial cells (31) but not in podocytes (60). It is also expressed in proximal tubular HK-2 cells (7).

TSP1 and CD47 in Renal Disease

TSP1 plays a role in several preclinical models of renal injury and disease. In renal fibrosis, TSP1 expression is increased and localized to tubular epithelial cells and peritubular interstitium (92). In a rat model of deoxycorticosterone acetate/salt-induced tubulointerstitial fibrosis, TSP1 was upregulated in cortical tubular cells (52), and in mice treated with mercuric chloride subsequent tubular injury and tubulointerstitial fibrosis were associated with increased TSP1 localized in tubular epithelial cells and the peritubular interstitium (92). TSP1, in part through binding and activation of the latent form of transforming growth factor-β (TGF-β1), induces progressive renal fibrosis (19, 35). In addition to inducing collagen and other matrix proteins associated with fibrosis, TGF-β1 is a potent inducer of TSP1 gene expression in mesangial cells (56), creating a positive feedback loop. Chronic glomerulonephritis patients with marked nephrosclerosis exhibit higher TSP1 in the renal interstitium (91). A peptide that inhibits TSP1-mediated activation of latent TGF-β1 decreases renal fibrosis in rats (97). Other peptides derived from the type I repeats of TSP1 also inhibit renal cell proliferation in an anti-Thy1-induced glomerulonephritis rat model independent of latent TGF-β1 activation and decreased proteinuria (36).

High glucose concentrations increased TSP1 in proximal (102) and distal (99) tubular epithelial cells. In a diabetic endothelial NO synthase (eNOS) null mouse model, high glucose levels correlated with renal tubulointerstitial injury and elevated TSP1 expression, while insulin corrected the renal injury (57). Conversely, activation of the canonical NO pathway with a phosphodiesterase (PDE) 5 blocker to increase the NO second messenger cGMP inhibited anti-Thy1-induced proliferative glomerulonephritis and lowered TSP1 in rats (33). In a streptozotocin-induced model of diabetic nephropathy, a PDE inhibitor suppressed TSP1 expression and ameliorated kidney injury (95), and exogenous NO decreased TSP1 production in cultured endothelial cells (83). Also, in a mouse model of diabetic nephropathy, the LSKL sequence from the latency-associated peptide, which inhibits TSP1-mediated latent TGF-β1 activation, improved renal function (62). Finally, in kidney biopsies from human subjects with diabetic nephropathy both glomerular and cortical TSP1 expression was associated with disease (32).

TSP1 mRNA was significantly increased in a porcine model of non-heart-beating donor renal transplantation (61). Interestingly, analysis of human renal allografts with chronic nephropathy demonstrated increased TSP1 expression (34). In mice and rats, TSP1 was upregulated in the proximal renal tubules following IRI, whereas TSP1 null mice were protected from renal injury (93). Similarly, we have reported hepatic induction of TSP1 in a murine model of warm subtotal liver IRI injury and protection from tissue injury and enhanced organ reperfusion in TSP1 null animals (45).

Less is known of the role of CD47 in renal pathophysiology. In renal diseases including acute postinfectious glomerulonephritis (GN), membranoproliferative GN, and diabetic nephropathy, CD47 expression decreased in mesangial cells (31). CD47 is also markedly decreased during mesangiolyis, but increased in mesangial cells in the restoration stage (60). In mice treated with ferric nitritolriacetate, CD47 is overexpressed in renal proximal tubular epithelial cells (RTEC) and in many of the secondary renal tumors (75). Interestingly, CD47 expression is decreased in circulating platelets in infection-mediated hemolytic uremic syndrome, and this process can be inhibited with an antibody to toll-like receptor 4 (30).

Activated CD47 Inhibits Multiple Cell Survival Pathways

Low (picomolar to nanomolar) concentrations of NO play a central role in cardiovascular physiology to promote arterial dilation and as an antithrombotic and anti-inflammatory agent (37). Work in our group has recently defined a novel regulatory role for TSP1, through binding and activating CD47, to redundantly inhibit the NO pathway (41, 43) (Fig. 1). At the level of endogenous NO production by eNOS, TSP1-CD47 signaling limits eNOS activity through suppressing calcium flux (5). Interestingly, null animals lacking endogenous TSP1 display enhanced eNOS activity under baseline conditions, as measured by eNOS phosphorylation (6). The primary intracellular target of NO is the enzyme soluble guanylyl cyclase (sGC) that, on binding NO via its prosthetic heme, increases the production of cGMP (38, 70). Activated CD47 directly inhibits NO-mediated stimulation of sGC (47), again in part by altering cellular calcium signaling (82). Activated CD47 also limits NO-independent sGC stimulation by non-heme-targeting chemical agents (71). In platelets and vascular smooth muscle cells, TSP1 inhibits downstream of sGC at the cGMP targetPKG (42) (Fig. 1). Results in vascular cell culture systems translate to in vivo effects, where soluble TSP1, by binding and activating endothelial CD47, inhibits arterial vasodilation and potentiates vasoconstriction (5, 42, 46). Activated CD47 expressed on platelets can further impede blood flow and tissue perfusion by inhibiting NO to stimulate platelet aggregation (51) and thrombosis.
cAMP is another important cellular messenger that promotes cell survival through regulation of multiple pathways (12). In the cardiovascular system, cAMP is a potent vasodilator of arteries and critical to myocardial function (103). In the kidney, cAMP has been linked to electrolyte transport (27). Recently, we determined that TSP1 can inhibit the production of cAMP by adenylyl cyclase in vascular smooth muscle cells (100) (Fig. 1), most likely in a CD47-mediated fashion (26, 65), consistent with our previous finding of increased basal myocardial cAMP levels in TSP1 and CD47 null mice (46).

Maintenance of a mature and healthy vascular network and adequate tissue perfusion requires the complex interaction of numerous mechanical and hormonal cues. A major role in this capacity has been ascribed to VEGF (17). Extending earlier reports from our group suggesting that TSP1 could limit VEGF-mediated effects on vascular cells (48), we recently reported that in endothelial cells CD47 constitutively associates with and binds to the VEGF receptor 2 (VEFR2) and that TSP1 binding to endothelial CD47 disrupts this association to inhibit VEGFR2 activation and VEGF signaling (54) (Fig. 1).

Mitochondria are specialized organelles that produce ATP, the primary chemical energy in eukaryotic cells. NO is a canonical nitric oxide (NO) pathway, limiting endothelial NO synthase (eNOS) activation (5), soluble guanylyl cyclase (sGC) stimulation (48), and the downstream cAMP target PKG (51). Thrombospondin-1 (TSP1) activation of CD47 decreases VEGF signaling by altering CD47 and VEGF receptor (VEGFR2) coassociation (54), to limit both NO-dependent and -independent effects of VEGF. TSP1, presumably via CD47, inhibits adenylyl cyclase to limit cAMP production (100). CD47 also limits mitochondrial energetics and promotes reactive oxygen species (ROS) and programmed cell death (26).

Activated CD47 Stimulates Multiple Cell Injury Pathways

Activation of CD47 induces cell death in neuronal cells (88) and breast carcinoma, multiple myeloma, and leukemic cell lines (98). However, the physiological relevance of these findings is unclear as antibodies were typically used to activate CD47, or supraphysiological concentrations of the TSP1-based peptide 4N1K were employed in these experiments as a surrogate for TSP1. Cell death stimulated by CD47 ligation can be caspase dependent or independent (66). Conversely, CD47-deficient T cells are resistant to Fas-mediated apoptosis in vitro and in vivo during resolution of a delayed type hypersensitivity response (64) (Fig. 1). Consistent with these reports, we have found that TSP1 and CD47 null vascular cells and tissues do not undergo cell death in response to high-dose radiation (44). CD47-mediated cell and tissue death under radiation stress appears to occur in part by activation of apoptosis. Conversely, highly proliferative tissues such as bone marrow are protected from radiation-stimulated cell death when CD47 activation is blocked with a morpholino oligonucleotide that suppresses protein production (68). Thus activated CD47 promotes radiation-mediated cell and tissue death.

ROS potentiate many acute and chronic diseases. Under hypoxia, TSP1 and CD47 are upregulated and associated with increased vascular cell production of ROS (6). Conversely, blocking CD47 activation with a monoclonal antibody in hypoxic endothelial cells completely abrogated ROS production, suggesting a direct role for activated CD47 in promoting vascular ROS production.
CD47 in IRI

IRI is a major cause of transplant dysfunction and failure (89) and plays a critical role in morbidity and mortality resulting from myocardial infarction (101) and stroke (21). The process of organ procurement for transplantation induces acute effects that decrease tissue blood flow and perfusion and secondary late effects that activate immune inflammatory pathways (28). In a cerebral IRI model, CD47 null mice were protected with less infarct and secondary brain swelling (53). We have reported that CD47 null mice and wild-type animals treated with antibodies that block CD47 activation by TSP1 are protected from soft tissue (67) and liver IRI (45). Interestingly, CD47 blockade imparts protective effects from IRI, even if therapy is started some time after reperfusion (67).

Activated CD47 in Acute Renal Injury

It was not known whether signaling through CD47 regulates IRI responses in visceral organs other than the liver. Furthermore, the mechanism through which activated CD47 promotes IRI, and specifically renal IRI, remains unknown. To investigate this, we challenged male C57BL/6 wild-type and CD47 null mice with bilateral renal ischemia followed by 24 h of reperfusion. We found upregulation of TSP1 and its principal receptor CD47 in wild-type kidneys following IRI (86). Conversely, in CD47 null kidneys, TSP1 protein and mRNA expression was significantly reduced compared with wild-type. RTEC are a primary target of IRI (22), with the death of these cells a prominent finding in human renal transplants (58). Under hypoxia and reoxygenation (30 min of 1% O2, followed by 24 h of normoxia as a mimic of renal IRI), RTEC show persistent induction of both TSP1 and CD47. Conversely, we (6) and others (77) have shown that in the presence of elevated glucose, hypoxia upregulates TSP1 in vascular endothelial and smooth muscle cells, respectively, and as we recently reported (86), in RTEC this process is resistant to oxygen-mediated downregulation.

Preclinical animal models of bilateral renal IRI induce a robust stress that results in animal death. Importantly, CD47 null animals challenged with renal IRI survived indefinitely whereas all wild-type animals had expired by 50 h (Fig. 2A).

![Fig. 2. Absence of CD47 activation provides significant survival advantage and renal cytoprotection following ischemia-reperfusion injury (IRI) in null mice. A: wild-type (WT) C57BL/6 male mice invariably died within 50 h following renal IRI. In contrast, similarly challenged CD47-- mice all survived as highlighted by Kaplan-Meyer analysis. B: WT mice subjected to 24-h reperfusion following bilateral renal ischemia demonstrated marked corticomedullary damage, characterized by widespread tubular epithelial cell necrosis (arrows) and cast formation (arrowheads). CD47-- mice had minimal alterations in cytoarchitecture on histological examination. Representative images of kidneys from both mice are shown (periodic acid-Schiff-stained; original magnification ×200, with insets at ×400).](http://ajprenal.physiology.org/doi/10.1152/ajprenal.00359.2012)
Enhanced survival following IRI in CD47 null mice correlated with less evidence of infarction, tubular necrosis, and cast formation compared with wild-type controls (86) (Fig. 2B). Serum creatinine, a biomarker of renal function, was significantly lower in CD47 null animals compared with wild-type mice following IRI. Caspase 3 activation stimulates both apoptotic and necrotic cell death and is a target for therapeutic mitigation of IRI (24). Wild-type mice demonstrated a significant increase in renal caspase 3 following IRI. In contrast, CD47 null animals showed minimal to no increase in renal caspase 3 (86), suggesting CD47 promotes apoptotic cell death in renal IRI.

CD47 is ubiquitously expressed, and it was not clear whether CD47 in the parenchyma or in circulating cells promoted renal injury. To determine this, we created bone marrow (BM) transplant chimeras and subjected them to renal IRI. CD47 null mice receiving wild-type BM experienced minimal to no tissue injury and markedly less increase in serum creatinine following IRI (86). In contrast, wild-type mice receiving wild-type BM experienced substantial tissue injury commensurate with significantly larger increases in serum creatinine following renal IRI. CD47 null mice that received CD47 null BM displayed slightly greater protection against renal IRI than null mice that received wild-type BM, although the difference did not reach statistical significance. Thus, in renal IRI, parenchymal CD47, and not leukocyte CD47, predominantly promotes tissue injury.

Oxidative stress arising from elevated ROS production underlies the pathophysiology of numerous cardiovascular maladies (3, 63, 69, 81, 105), including IRI (18), and is a robust stimulant of renal transplant rejection (80). ROS and associated oxidative stress can induce cell death (87), activate redox-signaling pathways in the vessel wall, and induce vascular dysfunction. We analyzed oxidative stress in kidneys from animals following IRI. CD47 null tissues demonstrated significantly less ROS at 24 h following IRI compared with wild-type mice (86). Pathological ROS alters proteins, adversely impacting their function (90). Under conditions of increased ROS, tyrosine residues in proteins undergo nitration (78, 104).

We found significant increases in 3-nitrotyrosine expression in wild-type kidneys following IRI. However, there was minimal evidence of this process in kidneys from CD47 null mice (86) indicative of decreased oxidative stress.

Inducible NO synthase (iNOS) is a major source of cellular ROS (59), and wild-type kidneys demonstrate robust induction of iNOS mRNA following IRI (86). In contrast in CD47 null mice...
kidneys, iNOS induction did not occur post-IRI. Inflammatory cells, particularly macrophages, are a major source of iNOS expression. CD47 null kidneys display decreased inflammatory cell invasion following IRI, which may in part explain the decrease in iNOS expression noted in null renal tissues. Alternatively, iNOS is reported to be regulated by caveolin-1 (Cav-1) (25), and we recently reported that Cav-1 is a target of CD47 in pulmonary arterial endothelial cells (6), although it is not clear whether a similar mechanism applies in the kidney.

The protective effect observed in CD47 null mice following IRI may in part be secondary to the identified role of CD47 in acute regulation of blood flow (50). Animals were challenged with 22 min of ischemia to the renal pedicle and 30 min and 24 h of reperfusion. Interestingly, renal blood flow following 30 min of reperfusion, as measured by laser Doppler, was comparable between wild-type and CD47 null mice but lagged markedly by 24 h in wild-type kidneys. In contrast, CD47 null renal perfusion approached preinjury levels at 24 h post-IRI.

Although results in CD47 null mice and chimeras identify a significant role for parenchymal CD47 in promoting renal IRI, clinical implications were unclear. To examine this, we prevented activation of CD47 by treating wild-type mice with a monoclonal antibody previously shown to provide tissue protection in vivo (50). Consistent with increased overall survival and renal preservation in the absence of CD47, mice treated with this CD47 antibody experienced decreased renal tubular injury (Fig. 3A), lower serum creatinine, and decreased proinflammatory cytokine and chemokine transcript levels (86). At the same time, antibody-treated mice displayed decreased expression of both TSP1 and CD47. This finding is interesting in light of our report that basal and hypoxia-stimulated CD47 expressions are markedly suppressed in TSP1 null endothelial cells (6) and suggests possible cross talk at the gene level between TSP1 and its cognate receptor CD47.

Overall, these data demonstrate that the TSP1–CD47 axis is upregulated in a preclinical model of renal IRI and that activated parenchymal CD47 promotes tissue injury and renal dysfunction via multiple mechanisms including inhibiting NO signaling to limit reperfusion and blood flow following renal IRI. Activated CD47 also potentiates renal tissue injury through activation of iNOS and increased production of pathological ROS (Fig. 3B). Together, these data point to CD47 as a redundant promoter of renal IRI and unique therapeutic target.

Future Directions

The secreted protein TSP1 has been linked to kidney disease in animal models although the clinical importance of these findings remains to be determined. It is even less clear what role activated CD47, as one of the cognate receptors for TSP1, plays in kidney disease. New studies now identify activated parenchymal CD47 as a proximate promoter of renal IRI through several mechanisms. In translational experiments, an antibody that blocks TSP1-mediated activation of CD47 protects animals from renal IRI. Delivery of a humanized version of this CD47 antibody as a component of standard transplant preservation fluid could provide enhanced protection of donor organs, especially those from expanded criteria donors, from transplant IRI. Such an approach may increase the number of suitable organs for transplantation. Although preliminary studies have tested responses to IRI, it will be important to determine whether blockade of CD47 activation can provide organ protection in other types of injury and what role CD47 targeting therapies may have in chronic renal disease.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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