Role of the JAK/STAT signaling pathway in diabetic nephropathy

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Marrero, Mario B., Amy K. Banes-Berceli, David M. Stern, and Douglas C. Eaton. Role of the JAK/STAT signaling pathway in diabetic nephropathy. Am J Physiol Renal Physiol 290: F762–F768, 2006; doi:10.1152/ajprenal.00181.2005.—Excessive cellular growth is a major contributor to pathological changes associated with diabetic nephropathy. In particular, high glucose-induced growth of glomerular mesangial cells is a characteristic feature of diabetes-induced renal complications. Glomerular mesangial cells respond to traditional growth factors, although in diabetes this occurs in the context of an environment enriched in both circulating vasoactive mediators and high glucose. For example, the vasoactive peptide ANG II has been implicated in the pathogenesis of diabetic renal disease, and recent findings suggest that high glucose and ANG II activate intracellular signaling processes, including the polyol pathway and generation of reactive oxygen species. These pathways activate the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascades in glomerular mesangial cells. Activation of the JAK/STAT signaling cascade can stimulate excessive proliferation and growth of glomerular mesangial cells, contributing to diabetic nephropathy. This review focuses on some of the key elements in the diabetic microenvironment, especially high glucose and the accumulation of advanced glycooxidation end products, and considers their impact on ANG II and other vasoactive peptide-mediated signaling events in vitro and in vivo.

ANG II: high glucose; glomerular mesangial cells; advanced glycooxidation end products

Renal disease is one of the leading causes of morbidity and mortality in patients with diabetes mellitus, and ANG II has been implicated in the pathogenesis of maladaptive growth in renal tissues in these patients (16, 28, 38–40, 52, 55, 60, 64). For example, inhibition of ANG II-induced growth of glomerular mesangial cells with angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARB) reduces renal disease in both diabetic patients and animal models (23, 69). Several highly relevant ANG II-induced renal responses, such as growth, contraction, and AT1 receptor density, are induced by high glucose. Furthermore, high glucose is closely associated with glomerulosclerosis in type 1 diabetes (39, 40, 64).

ANG II SIGNALING PATHWAYS

The ANG II receptor has been linked to many growth signaling pathways, including those involving JAK/STAT, p21ras/Raf-1/MAP kinase, and PLC-γ1 (Fig. 1). In this context, investigators and clinicians have been able to prevent both vascular and kidney cell growth by inhibiting many of these ANG II-mediated events using molecular, biochemical, and pharmacological approaches (9). The actions of ANG II are mediated through two principal types of cell surface receptors, AT1 and AT2, and most of the physiological responses to ANG II in glomerular mesangial cells occur via the AT1 receptor subtype (9, 41). The activation of AT1 receptors by ANG II results in G protein-mediated signaling, which includes PLC-dependent activation of PKC and release of calcium from intracellular stores (9). In addition, AT1 receptors also activate signaling path-
ways traditionally associated with growth factor receptors (i.e., the EGF receptor) and cytokine receptors (i.e., the IL-6 receptor). The AT1 receptor shares with growth factor and cytokine receptors the ability to induce expression of early growth response genes independently of de novo protein synthesis. These early response genes appear to be regulated posttranslationally via modification of a pool of preexisting transcription factors (50, 53, 54, 66). Therefore, intracellular signal transduction pathways directly control the ANG II-induced expression of these early growth response genes. Traditionally, three intracellular signaling pathways have been implicated in the activation of protooncogenes: the JAK/STAT, p21ras/Raf-1/MAP kinase, and the PLC-\(\gamma\) cascades (9, 41, 56). Agonist-stimulated AT1 receptors activate all three of these signal transduction pathways but with vastly different time courses: from seconds (activation of PLC-\(\gamma\)1 and the generation of inositol phosphates and calcium), to minutes (MAP kinase activation), to hours (JAK/STAT activation) (9, 56). However, the exact mechanism(s) by which the AT1 receptor couples to several different downstream signal transduction pathways with different time courses is not completely clear. Presumably, this involves a complex series of steps that selectively recruit, activate, and then inactivate each signaling system in a time-dependent manner.

**ROLE OF THE JAK/STAT PATHWAY IN ANG II SIGNALING**

This review focuses on one of these ANG II-induced signaling pathways, the JAK/STAT pathway, as a key mechanism for modulating renal cell growth. The JAK proteins are a family of cytosolic tyrosine kinases, which originally were thought to be coupled exclusively to cytokine receptors, such as those for the interleukins and interferons. The family contains four members (JAK1, JAK2, JAK3, and TYK2) (17, 58). In response to ligand binding to cytokine receptors, these JAK tyrosine kinases associate with, tyrosine-phosphorylate, and activate the cytokine receptor itself. Once activated, JAKs also tyrosine-phosphorylate and activate other signaling molecules, including the STAT family of nuclear transcription factors after binding of the STATs to the receptor (17, 58). Thus the JAK/STAT pathway is an important link between cell surface receptors and nuclear transcriptional events leading to cell growth. For example, STAT1, STAT3, and STAT5 are tyrosine-phosphorylated following exposure to ANG II in cardiac fibroblasts and AT1 receptor-transfected Chinese hamster ovary cells (10, 11, 47). Our group and others have shown that the JAK/STAT cascade can be activated by ANG II in vascular smooth muscle cells (VSMC), resulting in tyrosine phosphorylation of JAK2, STAT1, and STAT3 and the translocation of STAT1 and STAT3 to the nucleus (11, 42, 43, 45). ANG II exposure also stimulated the phosphorylated monomeric STAT proteins to form homodimer (STAT12, STAT32, or STAT52) or heterodimer (STAT1:STAT3) complexes referred to as sis-inducing factors (SIF). These SIF complexes subsequently translocate to the nucleus and interact with specific DNA motifs called sis-inducing elements (SIE) or prolactin-inducing element (PIE)-like elements within the c-fos promoter, culminating in the activation of this early growth response gene (10, 17, 47, 58).

Furthermore, we have previously shown that the COOH-terminal tail of the AT1 receptor binds to JAK2 in an ANG II-dependent manner (2). We have found that inhibition of
tyrosine phosphorylation, either with a pharmacological JAK2 inhibitor, AG-490, or by electroporation of blocking antibodies against STAT1 or STAT3, blocks ANG II-induced VSMC proliferation and DNA synthesis (43). These results indicate that G protein-coupled receptors, in particular the AT1 receptor, can operate via the same intracellular tyrosine phosphorylation pathways previously linked to cytokine and growth factor receptors. Finally, we have also demonstrated that the Src-homology tyrosine phosphatases, SHP-1 and SHP-2, have opposite roles in ANG II-induced JAK2 phosphorylation. SHP-1 appears to act as a conventional phosphatase, promoting JAK2 dephosphorylation and termination of the ANG II-induced JAK/STAT signaling. On the other hand, the role of SHP-2 is more complicated. Despite being a phosphatase, it seems to play an essential role in promoting JAK2 phosphorylation and initiation of the ANG II-induced JAK/STAT cascade, leading to cell proliferation. The motif in the AT1 receptor that is required for association with JAK2 is also essential for interaction with SHP-2 (46). Furthermore, SHP-2 is required for a JAK2-ANG II-AT1 receptor association (46). Thus SHP-2 may function as an adaptor protein for the JAK2 association with the receptor, thereby facilitating JAK2 phosphorylation and activation (46).

A ROLE FOR HIGH GLUCOSE IN ANG II-MEDIATED RESPONSES

A basic mechanism underlying diabetic nephropathy appears to be high glucose-induced production of TGF-β and extracellular matrix molecules like fibronectin (33, 49). For example, glomerular mesangial cells cultured in the presence of high glucose produce increased levels of TGF-β and extracellular matrix compared with normal ambient glucose (33, 49). In addition, high glucose increases de novo synthesis of the PKC activator diacylglycerol (19). Thus one mechanism accounting for high glucose-induced glomerular mesangial cell expression of TGF-β and extracellular matrix molecules may be chronic activation of one or more isoforms of PKC (31). However, other mechanisms have been suggested. For example, nonenzymatic modification of macromolecules to form advanced glycation end products (AGE), changes in sorbitol and myo-inositol metabolism, increased formation of oxidants, and activation of MAP kinase pathways might also be involved (24, 25).

Our group has found that high glucose increases both basal and ANG II-induced vascular smooth muscle cell growth, tyrosine phosphorylation, and AT1 receptor-JAK2 complex formation, as well as the extent of tyrosine and serine phosphorylation of STAT1 and STAT3 (5). We have also found that high glucose increased the ANG II-induced tyrosine phosphorylation and activity of SHP-1. On the other hand, high glucose increased the ANG II-induced tyrosine phosphorylation and activity of SHP-2 (5). These results suggest that increased and/or decreased activation of a tyrosine kinase (JAK2), tyrosine phosphatases (SHP-1 and SHP-2), and downstream transcription factors, such as STAT1 and STAT3, may be key mechanisms underlying ANG II-induced vascular smooth muscle cell growth in the presence of high glucose. Recent studies from our laboratory have also shown that high glucose augments the ANG II-induced JAK/STAT pathway in glomerular mesangial cells (4) and that this pathway contributes to high glucose-mediated expression of TGF-β and the extracellular matrix proteins collagen IV and fibronectin (70).

AGE, JAK2 ACTIVATION, AND THE KIDNEY

The formation of AGE is accelerated under conditions associated with oxidant stress, delayed turnover of macromolecules, and high levels of aldoses, as occurs in diabetes mellitus. One pathway for AGE formation is the interaction of an aldose with free NH2 groups on proteins, and the subsequent “Amadori rearrangement,” leading to formation of complex glycooxidized adducts known as AGE (59). AGE adducts are found in the plasma, intracellularly, on the cell surface, and in the extracellular space. The accumulation of AGE in the vasculature and kidney of patients with diabetes has been described in many studies (55, 59).

Accumulation of AGE in renal tissue alters the structural integrity of the glomerular basement membrane and extracellular matrix associated with mesangial cells and podocytes through formation of cross-links and protein accumulation (72). AGE have been implicated in the progression of diabetic complications because inhibition of their formation reduces diabetic complications (72). For example, prevention of AGE formation using aminoguanidine or blocking AGE-induced cross-linking prevents diabetic nephropathy in experimental animals (36). However, in each case, the mechanism of action of these agents is complex, and simple cause-effect relationships, with regard to the pathogenic potential of AGE, are difficult to ascertain. With respect to the cellular effects of AGE, investigators have suggested that the interaction of these nonenzymatically glycated adducts with the receptor for AGE (RAGE) contributes to diabetic complications. In the kidney, AGE-mediated activation of RAGE recruits and activates inflammatory cells and stimulates expression of TGF-β. The latter is known for its ability to stimulate production of extracellular matrix, including basement membrane thickening, mesangial expansion, and fibrosis. These events are closely tied to the pathogenesis of glomerular sclerosis and renal dysfunction. Binding of AGE to RAGE also stimulates expression of VEGF in podocytes, which likely increases glomerular permeability, leading to proteinuria. AGE-modified proteins in the glomerular filtrate are likely to bind to RAGE, the latter expressed in proximal tubules, and cause epithelial-myoﬁbroblast transdifferentiation and inﬂammation, via an NF-κB-dependent mechanism (36, 72). Therefore, the presence of AGE adducts in the glomerular filtrate has been suggested as a factor contributing to tubulointerstitial inﬂammation and accelerated renal dysfunction.

Consistent with a role for RAGE in diabetic nephropathy, homozygous RAGE null mice rendered diabetic display a protective phenotype; their kidneys do not show elevated levels of VEGF and TGF-β and do not display increased mesangial matrix and thickening of the glomerular basement membrane seen in diabetic wild-type mice. In addition, transgenic mice overexpressing human RAGE driven by the endothelial cell promoter Flk-1 have been generated and rendered diabetic (75). These animals develop accelerated glomerulosclerosis, and their urinary albumin excretion is about three times that of nontransgenic, nondiabetic mice. Podocytes normally express higher levels of RAGE than endothelial cells (72). The mild albuminuria of these diabetic RAGE transgenic mice (with
expression of the transgene targeted to the endothelium) may be present because the transgene is not expressed in podocytes, a key site of endogenous RAGE expression in glomeruli.

RAGE is a multiligand member of the Ig superfamily of cell surface receptors that interacts with pathogenic molecules implicated in inflammation, amyloidosis, and diabetes, as well as mediators potentially involved in homeostasis (59, 71). Engagement of RAGE by ligands triggers activation of key cell signaling proteins, such as p21ras and MAP kinases, at least in part by stimulating production of H2O2 and, thereby, reprogramming cellular properties. Recently, our group has shown in VSMC that 1) the RAGE ligand S100B augments ANG II-induced activation of the JAK/STAT pathway; 2) S100B-RAGE interaction induces intracellular production of ROS, such as H2O2; and 3) anti-RAGE IgG blocks S100B enhancement of ANG II-induced JAK2 tyrosine phosphorylation in VSMC. Moreover, we also found that PKC-β inhibition blocks S100B-induced production of H2O2 and that PLD modulation blocks S100B augmentation of ANG II-induced JAK2 tyrosine phosphorylation (61). These data lead us to hypothesize that in kidney cells (podocytes and glomerular mesangial cells), blockade of PKC-β and/or PLD will decrease and/or abrogate AGE-induced H2O2 production and, as a consequence, ANG II-induced activation JAK2 and STAT proteins.

**ROS PRODUCTION, JAK2 ACTIVATION, AND GLOMERULAR MESANGIAL CELL GROWTH**

As previously discussed, high glucose enhances ANG II-induced activation of the JAK/STAT pathway (5). In the context of diabetic complications, evaluating the molecular mechanisms responsible for modulating ANG II-induced activation of the JAK/STAT pathway in glomerular mesangial cells by high glucose is an important area. The mechanism(s) by which high glucose promotes JAK2 activation may be related to activation of JAK2 by ROS, and ROS are induced by high glucose in glomerular mesangial cells (24). For example, other investigators have shown that ROS stimulate the activity of JAK2 in both fibroblasts and A-431 cells (63). It has also shown that the activation of JAK2 by ANG II in rat aortic smooth muscle cells was significantly inhibited by the NADPH oxidase inhibitor diphenylene iodonium, consistent with the notion that ROS production contributes to JAK2 activation in response to ANG II (57). Therefore, these findings suggest that the JAK-STAT pathway responds to intracellular ROS and that the vasoactive peptide ANG II uses ROS as a second messenger to regulate JAK2 activation.

Recent studies from our laboratory have shown that high glucose, via the polyol pathway, induces a rapid increase in intracellular ROS, such as H2O2, which stimulates intracellular signaling events similar to those activated by ANG II, including phosphorylation of growth-promoting kinases such as JAK2 (62). The polyol pathway generates ROS (H2O2 and O2−) (13, 24), which can then act as signaling mediators in the activation of downstream mitogenic pathways, such as the JAK/STAT cascade (63). For instance, in VSMC, H2O2 has been shown to have an important role in regulating cell growth (68). It has also recently been reported that ANG II induces a rapid increase in intracellular H2O2, via NAD(P)H oxidase, which subsequently activates growth-related responses (68). Similar results have also been found for PDGF-induced cell proliferation, which was shown to be dependent on H2O2 (63). Furthermore, PDGF uses H2O2 as a second messenger to regulate activation of the JAK/STAT pathway in rat fibroblasts (63). Therefore, we hypothesize that high glucose augments the ANG II-induced JAK/STAT pathway and growth responses in glomerular mesangial cells through ROS (i.e., H2O2) generated via the polyol pathway.

**ROLE OF ANG II IN GLOMERULAR PATHOPHYSIOLOGY**

The AT1, but not the AT2, receptor subtype predominates in adult human and rat glomeruli and in glomerular mesangial cells (42, 44). In addition, the major physiological responses to ANG II are mediated through binding of the hormone to AT1 receptors. It has been demonstrated by a number of investigators that ANG II can function as a growth factor for cultured glomerular mesangial cells, inducing both hypertrophic and proliferative changes (74). Whole animal studies have also demonstrated mesangial cell proliferation following 2 wk of continuous ANG II infusion at 200 ng/min in the rat (67). In several animal models of chronic renal disease, in vivo administration of ACE inhibitors or ARB prevented mesangial cell growth and glomerulosclerosis (6, 8, 35, 37). However, it has been difficult in these studies to separate indirect effects of ANG II, i.e., secondary to hemodynamic changes, from more direct mesangial cell effects. However, ACE inhibitors and ARB have been shown to prevent the progression of human renal disease, in particular diabetic nephropathy, by abolishing compensatory glomerular hypertrophy in the absence of any hemodynamic effects (35).

**ROLE OF THE JAK/STAT PATHWAY IN GLOMERULAR PATHOPHYSIOLOGY**

In a recent study (7), our group has shown that high glucose altered activation of the JAK/STAT pathway in vivo through ANG II in rat kidney glomeruli by inducing phosphorylation of JAK2 kinase and STAT proteins, namely, STAT1, STAT3, and STAT5A/B. We also found that the streptozotocin (STZ)-induced diabetic rats treated with the JAK2 inhibitor AG-490 showed a significant reduction in urine output as well as a significant reduction in fluid intake. Furthermore, treatment with AG-490 lowered systemic blood pressure and significantly reduced urinary protein excretion. These are the first published observations we know of that demonstrate the activation of JAK2 in an in vivo model of glomerular dysfunction. In addition, we have also recently performed longer time course studies to further determine the profile of changes in ANG II-dependent JAK2 phosphorylation and proteinuria in this model of type I diabetes. We found that treatment with both candesartan and AG-490 prevented the development of both proteinuria and hypertension in the STZ model of diabetes for 4 and 12 wk (Banes-Berceli AK and Marrero MB, unpublished observations). These results provide further support for the hypothesis that the JAK2 contributes importantly to both acute and chronic glomerular dysfunction in diabetic nephropathy. Future studies will be needed to elucidate which STAT proteins are involved in both acute and chronic glomerular dysfunction in diabetic nephropathy.
ROLE OF ENDOTHELIN SIGNALING IN DIABETES

There is substantial literature linking endothelin with a spectrum of vascular diseases, including hypertension, atherosclerosis, and diabetes (21). For example, in diabetes, plasma levels of endothelin and cellular levels of ROS are significantly elevated (56). The association of ROS and endothelin in diabetes is well documented (20, 65). One source of endothelin in diabetes is likely to be endothelial activation. Vascular elaboration of endothelin promotes glomerular damage by augmenting vasoconstriction, glomerular permeability, mesangial cell proliferation, and extracellular matrix production (22). In support of this view, it has been reported that plasma endothelin antigen and vascular cell endothelin mRNA are increased in both humans with diabetes and in STZ-treated rats (27). Moreover, increased plasma endothelin levels in diabetes are associated with an elevated risk of microvascular complications, suggesting a possible link between endothelin and microangiopathy (1, 18). In addition, glucose is reported to be a potent stimulator of endothelin production from endothelial cells (1), implying that locally released endothelin may contribute to diabetic atherosclerosis and abnormal vasoregulation. The role of endothelin in diabetes has also been supported by the results of studies using the ETA receptor antagonist BQ-123 to treat STZ-induced diabetic rats. BQ-123 administration prevented renal disease and decreased production of extracellular matrix (26). Furthermore, endothelin interferes with insulin signaling in VSMC (29). Although these studies suggest a role for endothelin in diabetic nephropathy and atherosclerosis, considerable additional work will be necessary to provide support for such a link.

Intracellular signaling mechanisms for endothelin-mediated cellular activation are still being worked out, but some pathways have been elucidated. For example, endothelin induces VSMC proliferation through the p21ras/Raf-1/MAP kinase pathway (34). Additionally, endothelin activation of the Ca2+-regulated cytoplasmic proline-rich tyrosine kinase (PYK2) in C6 glioma cells involves phosphatidylinositol 3-kinase (32). Endothelin does not activate the JAK/STAT pathway in cardiac myocytes (48) or in human blood monocytes (14); however, it does in the Chinese hamster ovary cell line stably transfected with the ETα receptor (51). To date, there have been no studies investigating the ability of endothelin to activate the JAK/STAT pathway in kidney glomerular mesangial cells or kidney podocytes. Nevertheless, recent work from our laboratory has demonstrated that inhibition of the JAK/STAT pathway in vivo with the JAK2 inhibitor AG-490 prevents proteinuria in STZ-induced diabetic rats (7). Therefore, these results suggest that regulation and activation of the JAK/STAT pathway in many tissues and under disease conditions in vivo may present a novel therapeutic approach for treatment of complications in diabetes. Thus understanding how ET-1 can activate this important pathway and whether this pathway is altered in a disease state like diabetic nephropathy warrants further inquiry.

SIGNIFICANCE

Diabetic nephropathy complicates the course of diabetes mellitus in 30–40% of patients and is the most common cause of end-stage renal disease in the United States (12). Patients with diabetes account for almost 50% of those enrolled in the federally-funded end-stage renal disease program, at a cost of several billion dollars a year. Diabetic patients with nephropathy suffer a 100-fold greater risk of dying relative to the nondiabetic population. The need for research concerning the pathogenesis, prevention, and treatment of diabetic nephropathy is highlighted by the profound financial, social, and personal impact of this devastating complication. ANG II has been implicated in the pathogenesis of diabetic glomerular disease. This review has summarized the effects of the diabetic milieu (i.e., high glucose concentrations) on ANG II-mediated intracellular signaling events in vitro (cultured glomerular mesangial cells) and in vivo (rat kidney glomeruli). We hypothesize that high glucose and ANG II activate intracellular signaling processes, including the polyl pathway and generation of ROS, which, in turn, activate the JAK/STAT signaling cascades in glomerular mesangial cells. This signaling cascade then stimulates production of TGF-β and expression of collagen IV (Fig. 2). TGF-β is a potent cytokine known to increase synthesis of extracellular matrix proteins, such as collagen IV, that accumulate in the interstitium of the kidney capillary bed, resulting in a salient pathological finding of progressive diabetic nephropathy (15). Collagen IV is the principal extracellular matrix protein observed in the diabetic kidney, and its production is stimulated by high glucose, ANG II, and TGF-β (15). Therefore, our findings concerning involvement of the JAK/STAT pathway in glomerular pathology provide new insights into cellular and molecular mechanisms underlying early diabetic nephropathy. It is our anticipation that this work will provide a basis for novel diagnostic and therapeutic strategies aimed at preventing diabetic nephropathy.

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derived growth factor-induced vascular smooth muscle cell proliferation.


