Glomerular Localization and Expression of Angiotensin-Converting Enzyme 2 and Angiotensin-Converting Enzyme: Implications for Albuminuria in Diabetes

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Angiotensin-converting enzyme 2 (ACE2) expression has been shown to be altered in renal tubules from diabetic mice. This study examined the localization of ACE and ACE2 within the glomerulus of kidneys from control (db/m) and diabetic (db/db) mice and the effect of chronic pharmacologic ACE2 inhibition. ACE2 co-localized with glomerular epithelial cell (podocyte) markers, and its localization within the podocyte was confirmed by immunogold labeling. ACE, by contrast, was seen only in glomerular endothelial cells. By immunohistochemistry, in glomeruli from db/db mice, strong ACE staining was found more frequently than in control mice (db/db 64.6 ± 6.3 versus db/m 17.8 ± 3.4%; P < 0.005). By contrast, strong ACE2 staining in glomeruli from diabetic mice was less frequently seen than in controls (db/db 4.3 ± 2.4 versus db/m 30.6 ± 13.6%; P < 0.05).

For investigation of the significance of reduced glomerular ACE2 expression, db/db mice were treated for 16 wk with a specific ACE2 inhibitor (MLN-4760) alone or combined with telmisartan, a specific angiotensin II type 1 receptor blocker. At the end of the study, glomerular staining for fibronectin, an extracellular matrix protein, was increased in both db/db and db/m mice that were treated with MLN-4760. Urinary albumin excretion (UAE) increased significantly in MLN-4760–treated as compared with vehicle-treated db/db mice (743 ± 200 versus 247 ± 53.9 μg albumin/mg creatinine, respectively; P < 0.05), and the concomitant administration of telmisartan completely prevented the increase in UAE associated with the ACE2 inhibitor (161 ± 56; P < 0.05). It is concluded that ACE2 is localized in the podocyte and that in db/db mice glomerular expression of ACE2 is reduced whereas glomerular ACE expression is increased. The finding that chronic ACE2 inhibition increases UAE suggests that ACE2, likely by modulating the levels of glomerular angiotensin II via its degradation, may be a target for therapeutic interventions that aim to reduce albuminuria and glomerular injury.


A
ngiotensin-converting enzyme (ACE) is a monomeric, membrane-bound, zinc- and chloride-dependent peptidyl dipeptidase that catalyzes the conversion of the decapetide angiotensin I (AngI) to the octapeptide AngII by removing a carboxy-terminal dipeptide (1). ACE2 is a carboxypeptidase that preferentially removes carboxy-terminal hydrophobic or basic amino acids (2,3). ACE2 is the only known and enzymatically active homologue of ACE in the human genome (1). AngI and AngII, as well as numerous other biologically active peptides, are substrates for ACE2, but bradykinin is not (1). ACE2 cleaves AngI and AngII into inactive Ang 1-9 and the vasodilator and antiproliferative Ang 1-7 (4,5), respectively; therefore, ACE2 has the potential to counterbalance the effects of ACE (2,3). Whereas ACE is ubiquitously distributed, ACE2 is more tissue restricted. Initially, it was found to be restricted to heart, kidney, and testis (1), but more recently, it has been found also in lung and other tissues (6,7).

Studies in both rat and mouse kidney have shown the presence of ACE2 in renal tubules and glomeruli (8–10). In samples that were obtained from human kidney biopsies, ACE2 was found in glomerular epithelium and vascular smooth muscle cells of interlobular arteries (11).

There is growing interest in a possible role of ACE2 in diabetic kidney disease (9,10,12). Activation of the renin-angiotensin system (RAS) is widely believed to contribute to kidney injury in diabetes (13). We have suggested that ACE2, acting as a negative regulator of the RAS, may exert a renoprotective action (10). The pattern of ACE and ACE2 expression in renal cortical tubules of young db/db mice was characterized by low ACE but increased ACE2 protein (10). More recently, we found that these alterations in ACE2 protein in renal tubules from diabetic mice are accompanied by corresponding changes in enzymatic activity (12). In this study, we examined the localization of ACE and ACE2 in the glomerulus of control and diabetic mice. The glomerulus is the site of the nephron where the lesions of diabetic nephropathy appear earlier, and an increase in glomerular permeability is an early manifestation of diabetic kidney disease as reflected by the presence of albuminuria. Accordingly, we wanted to examine the localization of ACE2 within the glomerulus and also to test the hypothesis that downregulation of ACE2
activity may lead to worsening of albuminuria in diabetic mice.

Materials and Methods

Animals

Obese db/db mice (C57BLKS/J-db/db) were used as a model of type 2 diabetes, and their lean littermates (db/m) served as nondiabetic controls (Jackson Laboratory, Bar Harbor, ME). The db/db mouse is one of the best characterized and most extensively studied rodent models of type 2 diabetes (14). Heterozygous db/m littermates are lean and are spared from the induction of type 2 diabetes and its secondary complications (14). For this study, we used young (8 wk of age) female db/db mice to study an early phase of diabetes (3 to 4 wk of onset) without renal pathology by light microscopy (14). The Institutional Animal Care and Use Committee approved all procedures.

Tissue Preparation

Pentobarbital sodium was administered intraperitoneally, and kidneys were perfused with ice-cold PBS at a constant flow rate of 20 ml/min by using an infusion pump to flush out blood. Kidneys were removed quickly, cut longitudinally, and fixed with 10% buffered formalin phosphate (Fisher Scientific, Hanover Park, IL) for overnight. After paraffin embedding, tissue sections (4 μm) were deparaffinized in xylene and rehydrated through graded ethanol series.

Immunohistochemistry

Kidney sections (4 μm) were deparaffinized and rehydrated. Antigen retrieval was performed with a pressure cooker at 120°C in target retrieval solution (DAKO, Carpinteria, CA). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (Fisher Scientific). The primary antibodies and dilutions that were used in this study are as follows: Anti-ACE (1:2000; 5C4 gift of Dr. Sergei Danilov), anti-ACE2 (1:100; rabbit antibody [15]; a gift from Drs. M. Chapell and C.M. Ferrario, Wake Forest University, Winston-Salem, NC), and anti-fibronectin (1:400; Sigma-Aldrich, St. Louis, MO). Sections for ACE staining were washed with Tris-buffered saline with Tween-20 (DAKO) and incubated with biotinylated rabbit anti-rat IgG (Vector Laboratories, Burlingame, CA) followed by peroxidase-labeled streptavidin (DAKO). Sections for ACE2 and fibronectin staining were washed and incubated with goat anti-rabbit IgG conjugated with peroxidase-labeled polymer (DAKO). Peroxidase labeling was revealed using a liquid diaminobenzidine substrate-chromagen system (DAKO). Sections were counterstained with hematoxylin (Sigma) and dehydrated, mounted with Pertmount (Fisher Scientific), and coverslipped. Sections were examined and photographed with a Zeiss microscope. Nonimmune serum was used as control for specificity.

For assessment of the degree of ACE, ACE2, and fibronectin staining in glomerular tuft, a semiquantitative analysis of the immunoperoxidase stained sections was done as based on a pathologist-established score as follows, as described previously (16): 1 = no staining; 2 = weak staining; 3 = strong staining. Sections were examined blindly by three different observers, who assessed staining intensity of 100 glomeruli from each slide.

Immunofluorescence and Confocal Microscopy

The paraffin-embedded kidney sections (4 μm) were deparaffinized and rehydrated. After antigen retrieval, sections were permeabilized with 0.5% Triton-X100 for 5 min and blocked with 5% normal donkey serum in PBS for 1 h at room temperature. The sections were incubated with primary antibodies diluted in 2.5% donkey serum in PBS overnight at 4°C. Primary antibodies that were used for the immunofluorescence immunostaining were rat monoclonal ACE antibody (5C4; 1:100), our affinity-purified polyclonal ACE2 antibody (1:200), and one of the specific cell type markers. As podocyte markers, we used anti-nephrin (1:100; Santa Cruz Biotechnologies), which localizes specifically in the slit diaphragm (17); an antibody against synaptopodin (1:100; Santa Cruz Biotechnologies), which is an actin-associated protein in the podocyte foot process (18); and anti-podocin (diluted 1:100; Santa Cruz Biotechnologies), which is specific for the basal pole of podocyte along the glomerular basement membrane (19). Platelet-endothelial cell adhesion molecule (PECAM-1; CD31) antibody (1:100; Santa Cruz Biotechnologies) was used as an endothelial cell marker. Anti–α-smooth muscle actin antibody (1:200; Sigma) was used to stain mesangial cells. Sections were washed with Tris-buffered saline with Tween-20 three times and then incubated for 45 min with one of the respective secondary antibodies (Alexa Fluor 488 donkey anti-rat, Alexa Fluor 555 donkey anti-rabbit, Alexa Fluor 647 donkey anti-goat, and Alexa Fluor 647 donkey anti-mouse IgG; Molecular Probes, Eugene, OR) diluted 1:200 in PBS with 2.5% donkey serum. Sections were washed three times, coverslipped with Prolong Gold antifade reagent (Molecular Probes), and sealed with nail polish. Sections were visualized with a Zeiss LSM 510 confocal microscope (Carl Zeiss Microscopy, Jena, Germany). Negative controls for immunofluorescence staining were performed by substitution of nonimmune serum for the primary antibodies.

Immunogold Electron Microscopy

Kidney cortex was cut into 1-mm³ blocks and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C for overnight. Tissue blocks were postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h and dehydrated in graded ethanol. Blocks were infiltrated in LR white resin, transferred into gelatin capsules with resin, and polymerized at 60°C for 24 h. Immunolabeling was performed on ultrathin sections that were cut with an ultramicrotome and picked up on nickel grids. Sections were rinsed with PBS and incubated in blocking solution (Electron Microscopy Sciences, Hatfield, PA) for 30 min, rinsed, and incubated overnight at 4°C with rat anti-ACE (1:100) or rabbit anti-ACE2 antibody (1:200) diluted in PBS with 0.1% normal goat serum. After washing in PBS, sections were incubated for 1 h with goat anti-rat or goat anti-rabbit IgG coupled with 10 and 15 nm of gold particles (Electron Microscopy Science), respectively. The sections were postfixed with 2.5% glutaraldehyde, stained with uranyl acetate and lead citrate, and examined using a JEOL 1220 electron microscope. Images were captured with a Gatan digital camera.

Pharmacologic ACE2 Inhibition in db/m and db/db Mice

A specific ACE2 inhibitor, MLN-4760 (gift from Millennium Pharmaceuticals, Cambridge, MA), was injected into db/m and db/db mice subcutaneously (40 to 80 mg/kg body wt, every other day), starting at 8 wk of age until the mice reached the age of 24 wk. Vehicle control mice received injections of sterile PBS in the same volume. A group of db/db mice received both the AT1 receptor antagonist telmisartan (Boehringer Ingelheim, Ingelheim, Germany), in drinking water in a dose of 2 mg/kg body wt per d, and the subcutaneous injections of MLN-4760.

Urinary Albumin/Creatinine Ratio

To measure albumin/creatinine ratio in urine samples, ELISA kit for murine urinary albumin and creatinine companion kit from Excellor (Philadelphia, PA) were used according to the manufacturer’s instructions. Spot urine samples were collected at 8 wk of age, before initiation of the administration of the ACE2 inhibitor and the AT1 blocker (at 8 wk of age), and after 12 and 16 wk of administration of these agents.
Statistical Analyses

Statistical analysis was performed using unpaired t test or ANOVA when appropriate. Significance was defined as $P < 0.05$. Data are expressed as mean ± SEM.

Results

Localization of ACE and ACE2 Using Immunofluorescence and Confocal Microscopy

ACE and ACE2 co-localized strongly in the apical brush border of the proximal tubule (Figure 1A). ACE seemed to be restricted to the apical border, whereas ACE2 also was present, albeit weakly, in the cytoplasm (Figure 1A). Both ACE and ACE2 were present in the glomerulus, but in contrast to proximal tubules, there was no co-localization of ACE and ACE2 in the glomeruli (Figure 1B).

To localize further ACE and ACE2 within the glomerular structures, we used markers for epithelial, mesangial, and endothelial cells. ACE co-localized with PECAM-1 (an endothelial cell marker; Figure 2, top), whereas ACE2 did not (Figure 2, bottom). ACE did not co-localize with either nephrin (Figure 3, top), synaptopodin (Figure 4) or podocin. In contrast, ACE2 co-localized with nephrin (Figure 3, bottom), synaptopodin (Figure 4), and podocin (data not shown).

ACE2 also co-localized with α-smooth muscle actin, a marker of mesangial cells, whereas ACE did not (Figure 5). In summary, glomerular ACE2 co-localized with both podocyte and mesangial cell markers, whereas ACE did not. ACE co-localized with an endothelial marker, whereas ACE2 did not. The pattern of cell-specific distribution of glomerular ACE and ACE2 was similar in kidneys from db/m and db/db mice.

Glomerular ACE2 and ACE Localization by Immunogold Electron Microscopy

Immunogold electron microscopy labeling was used to study the ultrastructural localization of ACE2 and ACE in glomeruli from db/m and db/db mice. ACE labeled with gold particles was predominantly localized in podocyte foot processes (Figure 6A). ACE2 also was found in the body and slit diaphragm of the podocyte and, to a smaller extent, in mesangial cells (data not shown). In contrast to ACE2, ACE labeled with gold parti-
ACE and ACE2 Expression in Control and Diabetic Mice Kidneys

In glomeruli from 8-wk-old db/db mice, ACE staining was increased as compared with db/m (Figure 7, compare B with A). ACE2, by contrast, was decreased in db/db as compared with db/m (Figure 7, compare D with C). Strong staining was used to semiquantify the observed changes on the basis of data from kidneys from six animals in each group (see the Materials and Methods section). In diabetic mice, the percentage of glomeruli with strong ACE staining was increased as compared with glomeruli from control mice (db/db 64.6 ± 6.3 versus db/m 17.8 ± 3.4%; P < 0.005; Figure 7). In contrast to these findings with ACE, the percentage of glomeruli with strong ACE2 staining was reduced in diabetic mice in comparison with controls (db/db 4.3 ± 2.4 versus db/m 30.6 ± 13.6%; P < 0.05; Figure 7).

In kidneys from db/db mice, proximal tubular staining for ACE was less intense than in tubules from the db/m mice (Figure 7, compare B with A). By contrast, ACE2 staining in tubules from the db/db mice was increased as compared with the control db/m mice (Figure 7, compare D with C). This finding confirms our previous studies in tubules from the db/db mice (10). Like in proximal tubules, glomerular parietal epithelial ACE2 staining was increased in the db/db mice (Figure 7D). There was no ACE staining in parietal glomerular epithelium from either db/db or db/m mice.

Effect of Chronic ACE2 Inhibition on Albumin Excretion and Glomerular Fibronectin Deposition

To determine whether chronic ACE2 inhibition results in increased albuminuria in the db/db mice, we administered a specific ACE2 inhibitor, MLN-4760, for 16 consecutive weeks starting at 8 wk of age. At 8 wk of age, before starting MLN or vehicle administration, db/db mice from both groups had virtually indistinguishable levels of urinary albumin excretion (UAE; 69.5 ± 18 versus 81 ± 15 μg albumin/mg creatinine, respectively). As previously reported by others (14), we found that at this age, albumin excretion is already significantly higher in the db/db than in the db/m mice (81 ± 15 versus 45 ± 5 μg albumin/mg creatinine, respectively). In db/db mice that received MLN-4760, albumin/creatinine ratio was significantly higher than in their vehicle-treated counterparts already after 12 wk of treatment (474 ± 166 versus 124 ± 23 μg/mg, respectively; P < 0.05). After 16 wk of treatment, at the age of 24 wk, MLN-treated db/db mice had approximately three-fold increased UAE in comparison with vehicle db/db controls (743 ± 200 versus 247 ± 53.9 μg/mg, respectively; P < 0.05; Figure 8). In db/m mice that were treated with MLN-4760, UAE was higher than in the vehicle-treated db/m controls, but the difference was small and not statistically significant (55 ± 24 versus 32 ± 3 μg/mg, respectively; NS).

To study whether the effect of ACE2 inhibition is mediated via AngII and, more specific, the AT1 receptor, we gave both MLN-4760 and telmisartan to db/db mice. The administration of telmisartan to diabetic mice completely prevented the increase in urinary albumin that was associated with administration of the ACE2 inhibitor (Figure 8). This shows that the effect of ACE2 inhibition on albuminuria requires stimulation of the AT1 receptor presumably by increased levels of AngII.

Chronic ACE2 inhibition also was associated with increased glomerular deposition of fibronectin, an extracellular matrix protein (Figure 9). In glomeruli from db/m mice that

Figure 4. Triple immunofluorescence staining of ACE (green; A), ACE2 (red; D), and synaptopodin (blue; B and E), a podocyte foot process marker. ACE2 weakly co-localizes with synaptopodin (pink, arrows; F), whereas ACE does not co-localize with it (C).

Figure 5. Triple immunofluorescence staining of ACE (green; A), ACE2 (red; D), and α-SMA (blue; B and E). ACE and α-SMA do not co-localize in the glomeruli (C). ACE2 shows co-localization with α-SMA in some areas of the glomerular tuft (pink, arrows; F).
received MLN-4760, fibronectin staining was increased as compared with their vehicle-treated db/m counterparts (Figure 9, compare B with A). In db/db mice, the MLN-4760 administration also was associated with an exaggeration of fibronectin staining (Figure 9, compare D with C). The number of glomeruli with strong fibronectin staining was used to semiquantify the observed changes in kidneys from 12 to 16 mice in each group (see the Materials and Methods section). In db/m mice that received MLN-4760, the percentage of glomeruli with strong fibronectin staining was increased as compared with glomeruli from vehicle-treated db/m controls (41.1 ± 4.1 versus 17.3 ± 5.2%, respectively; \( P < 0.005 \); Figure 9). Similar to the findings in db/m mice, the percentage of glomeruli with strong fibronectin staining was increased in diabetic mice that were treated with MLN-4760 in comparison with the db/db mice that received vehicle (54.8 ± 4.6 versus 28.5 ± 6.4%, respectively; \( P < 0.005 \); Figure 9).

**Discussion**

This study shows that ACE and ACE2 strongly co-localize on the apical surface of the proximal tubules, whereas in glomeruli, ACE and ACE2 are present but do not co-localize. To identify ACE and ACE2 within distinct glomerular structures, we used subcellular and cell type–specific markers for immunofluorescence staining with confocal microscopy as well as immunogold labeling. ACE2 was expressed mainly in visceral
epithelial cells (podocytes) and in parietal epithelial cells of the Bowman’s capsule. Within the glomerular tuft, ACE2 co-localized with nephrin (a slit diaphragm protein), podocin (a marker of the basal pole of the podocyte), and synaptopodin (a marker of the podocyte foot process), indicating that ACE2 is present in the podocyte/slit diaphragm complex. ACE2 also co-localized, albeit not as strongly, with α-smooth muscle actin, which indicates its presence in mesangial cells. ACE, by contrast, did not co-localize with podocyte or mesangial cell markers. ACE co-localized with PECAM-1, reflecting its presence in glomerular endothelial cells. Immunogold studies further showed that ACE2 is present in podocyte foot processes and ACE in endothelial cells (Figure 6). ACE2 also was found in the body and slit diaphragm of the podocyte and, to a smaller extent, in mesangial cells. The glomerular localization of ACE and ACE2 by immunofluorescence and immunogold electron microscopy was similar in db/m and db/db mice. By immunohistochemistry, however, the pattern of strong glomerular ACE and ACE2 protein staining differed strikingly between db/db and their lean counterpart, the db/m mice, of the same age (8 wk). That is, strong ACE2 protein staining in glomeruli from diabetic mice was increased (Figure 7).

On the basis of our findings of glomerular cell specificity of ACE and ACE2, we can infer the glomerular sites that are accountable for the differences between db/m and db/db mice in terms of ACE and ACE2 expression that were observed by immunohistochemistry (Figure 7). Because ACE was expressed in endothelial cells of db/db and db/m mice but not in podocytes or mesangial cells, it is reasonable to conclude that the strong glomerular staining of ACE that was seen in the db/db mice reflects an increase at the level of glomerular endothelial cells. This is supported further by the fact that we found no evidence of aberrant expression of ACE (i.e., ACE outside endothelial cells) in the immunolocalization studies that were performed in the db/db mice. Conversely, because ACE2 was not present in endothelial cells of either db/db or db/m mice, the reduction in glomerular staining of ACE2 in the db/db mice likely reflects a decrease in protein expression at the level of the podocyte and possibly mesangial cells (or both).

The compound MLN-4760 is a specific ACE2 inhibitor that exerts its inhibitory action by binding to two metalloproteinase catalytic subdomains of the ACE2 enzyme (20). To examine the potential role of ACE2 enzyme in the development of albuminuria, we administered MLN-4760 for several weeks. This resulted in a significant increase in albumin excretion in the db/db mice (Figure 8). By 24 wk of age, albumin excretion was approximately three-fold higher in db/db mice that were treated with MLN-4760 as compared with vehicle-treated db/db controls. The specific AT1 blocker, telmisartan, prevented the increase in UAE that was associated with MLN-4760, suggesting that the effect of ACE2 inhibition is mediated by AngII via stimulation of the AT1 receptor (Figure 8). ACE2 inhibition also was associated with increased glomerular expression of fibronectin in both db/m and db/db mice (Figure 9). In normal kidney, fibronectin is present along the basement membranes. During glomerular injury, fibronectin deposition increases, and this increase is considered a marker of extracellular matrix accumulation (21). Glomerular fibronectin accumulation occurs as early as 7 d after AngII infusion (22). We think that MLN-4760, by inhibiting ACE2, leads to increased extracellular matrix deposition by promoting AngII accumulation within the glomerulus. Although we did not measure AngII levels after ACE2 inhibition, others have shown that in Ace2 knockout, AngII is either endogenously elevated (15,23) or increased above the levels of wild-type mice after infusion of exogenous AngII (24).

Our finding that ACE2 inhibition did not increase albumin excretion significantly in nondiabetic female mice is in keeping with the work of Oudit et al. (23) using an Ace2 knockout. These authors found that deletion of the Ace2 gene was associated with the development of albuminuria over time (12 mo of age) in male but not in female mice. In general, it is more difficult to produce albuminuria in female than in male mice (14,25). In this respect, it is noteworthy that in this study we used female db/db mice, because we were not aware of the findings of Oudit et al., which were published just recently (23). Our finding that in female mice ACE2 inhibition resulted in worsening of albuminuria further shows the importance of this enzyme in the control of the glomerular permeability. We surmise that in male mice, ACE2 inhibition would promote albuminuria to a further degree and that this would affect nondiabetic mice as well as diabetic mice, but this will await further studies. It also is of interest that ACE2 inhibition in female db/m mice did not result in significant albuminuria despite a significant increase in glomerular fibronectin staining, a marker of mesangial matrix deposition.

AngII impairs the function of glomerular barrier, leading to increased protein excretion, and agents that interfere with AngII activity, such as ACE inhibitors and AT1 blockers, reduce
filtration of macromolecules across the glomerular barrier (13,26–28). A recent study further showed that the abnormal protein efflux across the glomerular membrane could be mediated by AngII-induced actin cytoskeleton rearrangement in glomerular epithelial cells (29). We propose that the presence of ACE2 in the podocyte/mesangial compartment of the glomerulus could have an important counterregulatory role by preventing glomerular AngII accumulation (Figure 10). In this respect, the reduction in glomerular ACE2 that was observed in the young db/db mice could be deleterious because AngII degradation via ACE2 is apt to be decreased, particularly when coupled with increased AngII formation that is driven by augmented ACE activity in endothelial cells. It should be noted that the db/db mice at the age of 8 wk have no evidence of glomerular lesions by light microscopy, but at this early age, albumin excretion was already significantly higher in the db/db than in the db/m mice, as previously reported by Sharma et al. (14). This increase in albumin excretion reflects an increase in glomerular permeability related to changes in glomerular hemodynamics, subtle podocyte injury, or both (30). We suggest that down-regulation of ACE2 may play a role by reducing AngII degradation, whereas the increase in endothelial ACE activity further results in excess AngII. A cross-talk between podocyte and endothelial cells was proposed recently to explain the effect of vascular endothelial growth factor that is produced in the podocytes on glomerular endothelial permeability (31). It is possible that the effect of AngII on augmenting glomerular permeability involves increased vascular endothelial growth factor mRNA translation, as recently suggested (32,33).

It is known that there are AngII receptors (AT1) in glomerular epithelial cells (podocytes) and that AngII activates signal transduction pathways in these cells. The glomerular podocyte has a local RAS (34–36), and a recent study showed that mechanical stress increases AngII production in conditionally immortalized podocytes (37). Our study shows that ACE is not present in the podocyte, which is consistent with in vitro studies by Druvasula et al. (37) on immortalized podocytes showing no ACE-dependent AngII formation. We propose that whether the source of Ang peptides is systemic, from paracrine sources or locally generated within the podocyte, ACE2 could be critical in determining the levels of Ang peptides by promoting AngII degradation to Ang 1-7 and AngI degradation to Ang 1-9. Any direct role, if any, of these peptides in affecting glomerular permeability needs to be examined. Regardless of any potential effect of these peptides on glomerular permeability, a decreased expression of ACE2 protein and an increase in ACE

Figure 9. (Left) Immunohistochemistry of kidney sections from db/m (A and B) and db/db mice (C and D) showing an example of glomerular fibronectin staining after vehicle (A and C) or MLN-4760 administration (B and D). In vehicle-treated db/m mouse, the glomerulus shows slight fibronectin staining (reddish-brown; A), which is increased in a db/m mouse that received MLN-4760 (B). In an MLN-treated db/db mouse, there is a marked increase in glomerular fibronectin staining (D) as compared with a glomerulus from a vehicle-treated db/db mouse (C). (Right) The percentage of strongly stained glomeruli for fibronectin in vehicle-treated (□) and MLN-4760–treated mice (■).

Figure 10. A proposed scheme whereby excess glomerular angiotensin II (AngII) accumulation in diabetic nephropathy results from increased ACE and decreased ACE2 in the glomeruli.
favors AngII accumulation, which, in turn, would lead to increased glomerular permeability (Figure 10).

Glomerular ACE2 and, most specific, its presence within the podocyte/slit diaphragm complex normally could be protective against AngII-mediated increases in glomerular permeability. We suggest that ACE2 activity within the glomerulus exerts a renoprotective effect by favoring the rapid degradation of Ang peptides and thereby preventing exposure to high levels of AngII. This may be particularly relevant at the level of the podocyte, a cell that may not be programmed to tolerate AngII, which would be in keeping with the lack of ACE expression.

Our findings in the glomerulus are in sharp contrast with the findings in renal cortical tubules from db/db mice, where ACE staining is decreased but ACE2 is increased (Figure 7). The latter finding confirms our previous report (10). A decrease in tubular ACE was described originally by Anderson et al. (38) in streptozotocin-treated rats. Moreover, these authors suggested also that glomerular ACE was increased in this model of diabetes (38). There also have been reports of an increase in ACE in the glomerulus of patients with diabetes and nephropathy (16). An increase in ACE expression in glomerular endothelial cells from animals and humans with diabetes may be the result of the generalized endothelial dysfunction, which is recognized increasingly in early stages of diabetes. Hyperfiltration, which is present already at an early age in the db/db mice, could play an additional role at the level of the glomerular endothelium. We speculate that excessive ACE activation could be an important event in the activation of the RAS in diabetes and therefore play a more proximate role than generally appreciated. A primary role of ACE overactivity on diabetes-related renal injury can be inferred from studies in transgenic mice with three copies of the Ace gene (39). Transgenic mice with one, two, or three copies of ACE were studied after induction of diabetes with streptozotocin (39). After induction of diabetes, there was a moderate but significant increase in UAE in one- and two-copy mice but a much larger increase in the three-copy ACE mice (39). The overexpression of endothelial ACE coupled with the underexpression of ACE2 in podocytes and mesangial cells is a combination that is apt to increase AngII within the glomerulus (Figure 10).

Conclusion

Our study shows that ACE2 is present in podocytes and, to a lesser extent, in glomerular mesangial cells, whereas ACE, by contrast, is present only in endothelial cells. We propose that ACE2, by regulating the degradation of Ang peptides, prevents AngII accumulation in the glomerulus. Reduced glomerular ACE2 in the db/db mouse likely is deleterious by favoring AngII accumulation, leading to an increase in glomerular permeability early, and may foster progressive glomerular injury over time. Our finding of increased albuminuria in the db/db mice that were treated with an ACE2 inhibitor suggests a role of this enzyme in the regulation of Ang peptides and thus glomerular permeability. The possibility of therapies that are targeted to amplify glomerular ACE2 expression as a way to reduce proteinuria and confer renoprotection early in the course of diabetic nephropathy and possibly other kidney diseases needs to be investigated.

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