Glucose, Glycation, and RAGE: Implications for Amplification of Cellular Dysfunction in Diabetic Nephropathy

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Abstract. Receptor for advanced glycation endproducts (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules. Driven by rapid accumulation and expression of key ligands such as advanced glycation endproducts (AGE) and S100/calgranulins in diabetic tissues, upregulation and activation of RAGE magnifies cellular perturbation in tissues affected by hyperglycemia, such as the large blood vessels and the kidney. In the diabetic glomerulus, RAGE is expressed principally by glomerular visceral epithelial cells (podocytes). Blockade of RAGE in the hyperglycemic db/db mouse suppresses functional and structural alterations in the kidney, in the absence of alterations in blood glucose. Recent studies in homozygous RAGE null mice support a key role for RAGE in glomerular perturbation in diabetes. Importantly, beyond diabetes, studies in other settings of glomerulopathies support a critical RAGE-dependent pathway in podocytes linked to albuminuria, mesangial expansion, and glomerular sclerosis. A new paradigm is proposed in glomerular injury, and it is suggested that blockade of the RAGE axis may provide a novel means to prevent irreparable glomerular injury in diabetes and other sclerosing glomerulopathies.

The “Problem” of Glucose

Irrespective of the underlying mechanisms, elevated levels of blood glucose ignite a vicious cycle of metabolic disturbances within the intracellular and extracellular environment that, if left unchecked, lead to a broad array of complications in macrovessel and microvessel structures. A particular target in this setting is the kidney. Epidemiologic studies demonstrate that diabetes is a leading cause worldwide of end-stage renal failure requiring renal replacement therapy (1). A number of pathways are activated in the hyperglycemic milieu, such as the aldose reductase pathway, activation of protein kinase C, especially the \( \beta \)-isoform, and the generation of advanced glycation endproducts (AGE). Work from Dr. Michael Brownlee’s laboratory indicates that there is fundamental crosstalk among these pathways, as evidenced by the demonstration that normalization of mitochondrial superoxide dismutase, blocked each of these three pathways in cultured bovine aortic endothelial cells subjected to hyperglycemia-driven generation of reactive oxygen species (2).

In \textit{vivo}, the roles of each of multiple pathways implicated in the pathogenesis of diabetic nephropathy have been rigorously tested experimentally. Examples of these include activation of aldose reductase and protein kinase C, particularly the \( \beta \)-isoform. Importantly, however, roles for other pathways, such as activation of the family of mitogen-activated protein kinases (MAP kinases) and the transcription factor nuclear factor-\( \kappa B \) (NF-\( \kappa B \)) have been linked to the pathogenesis of hyperglycemia-mediated stress (3). Two examples of mediators of hyperglycemic stress will be addressed here.

First, roles for aldose reductase (AR) have been implicated in the adverse cellular response to high levels of glucose (4–5). Multiple studies indicate that expression and activity of aldose reductase is increased in experimental models and human tissues in diabetes, including the diabetic kidney (6–7). Pharmacologic inhibition of AR has implicated AR in the development of diabetes-associated nephropathy; for example, administration of the inhibitor epalrestat to streptozotocin-induced diabetic rats prevented the development of renal hypofunction and mesangial expansion without affecting levels
of blood glucose (8). Similarly, administration of tolrestat to streptozotocin-induced diabetic rats prevented glomerular hyperfiltration, mesangial cell hypocontractility, and enhanced permeability to albumin (9). Recent studies, however, have suggested that the role of AR in diabetic kidney disease may be more complex; Ng and colleagues showed that overexpression of human AR in transgenic rats prevented the development of albuminuria (10). One caveat in interpretation of the latter studies is that transgene expression was primarily localized to the outer strip of the outer medulla, compatible with the distribution of the pars recta of the proximal tubule. Thus, in the absence of glomerular expression of the transgene, it is difficult to conclude that AR played a solely protective role in this model. Nevertheless, these findings highlight the possibility that AR activity has distinct roles in different compartments of the diabetic kidney, components of which may be underscored by different strategies, such as pharmacologic blockade or transgene expression.

In addition to AR, the family of protein kinase C, consisting of multiple isoforms, especially PKCβ, has been associated with enhanced activity in hyperglycemia (11–12). Activation of these pathways has been demonstrated in glucose-treated glomeruli or mesangial cells, including activation of the transcription factor NF-κB (13). In vivo, pharmacologic blockade of PKCβ has been associated with improved vascular function in diabetic rats, as well as amelioration of accelerated mesangial expansion and expression of genes such as TGF-β, extracellular matrix components, and prostanooids in db/db mice, a model of insulin-resistant diabetes (14–16).

Importantly, recent studies have suggested a link among glucose-modified proteins, Amadori products and AGE, and activation of PKCβ isoforms. In cultured mesangial cells, inhibitors of PKCβ isoforms prevented the glycated albumin-induced increased expression of collagen IV (17). In other studies, Scivittaro et al. (18) showed that exposure of neonatal mesangial cells to AGE-rich proteins resulted in increased oxidative stress and increased intracellular calcium, in parallel with enhanced membrane translocation of the PKCβ isoform; in contrast, no change in localization of PKCα was identified in these studies.

These data highlight the concept that optimal management of diabetic complications will require the full dissection of the interplay between the direct and indirect consequences of elevated levels of glucose. Specifically, identification of the biochemical and molecular links by which elevated glucose triggers activation of these seemingly diverse pathways will undoubtedly highlight new targets for therapeutic intervention in the future. In the context of diseases of the kidney, nevertheless, experimental evidence supports the concept that AGE are linked to events that lead to scarring and functional disturbances in diabetes. Indeed, blockade of this axis in diabetic complications has been tested, both in experimental animals models and human subjects.

**Glucose, Oxidant Stress, and Formation of AGE**

The Maillard reaction has been identified as a key consequence of elevated levels of glucose. Although this process occurs in normal aging, there is little doubt that it is accelerated in diabetes (19–20). In the Maillard reaction, the amino groups of a protein react nonenzymatically with glucose to form a Schiff base. Schiff bases are temporarily stabilized through Amadori rearrangement and represent an early step in the glycation process. In the advanced step, complex reactions occur that lead to the formation of AGE. Both glucose-derived Amadori products and fructose are thought to be potential precursors of 3-deoxyglucosone (3-DG) in vivo. 3-DG can further react with proteins to form pyrallines or pentosidine (21–23). Other AGE, such as (carboxymethyl) lysine (CML)-modified adducts of proteins and lipids accumulate readily in vivo, and to date, have been identified as the most prevalent AGE species found in tissues (24–25). Further, the biologic implications of the formation of AGE, such as CML, have been expanded on the basis of the experimental observation that these adducts may be formed by the myeloperoxidase pathway, even in euglycemia (26).

In this context, numerous studies have documented that CML-AGE are present to elevated degrees in human diabetic kidney (27–28). These considerations led to the hypothesis that AGE might contribute, at least in part, to the pathogenesis of diabetic nephropathy. Accelerated AGE formation in diabetes likely eventuates in a vicious cycle whereby progressive renal dysfunction, delayed protein turnover, and accelerated oxidant stress contribute to aggressive formation of AGE (29–30). Unless interrupted, this cascade of events leads inexorably to renal failure.

Taken together, activation of these metabolic pathways in hyperglycemia, either by direct or indirect consequences of elevated levels of blood glucose, is linked to the pathogenesis of diabetic complications. Indeed, the results of the Diabetes Control Clinical Trials (DCCT) crisply elucidated that hyperglycemia is directly linked to diabetic microvascular complications, particularly in the kidney, as subjects treated with rigorous control of blood glucose displayed the greatest reduction in renal complications (31).

**AGE: Mechanisms Linked to Cellular Stress and Tissue Dysfunction**

**Receptor-Independent Pathways.** AGE cause cellular dysfunction by multiple mechanisms, including receptor-independent and receptor-dependent processes. In the former setting, AGE may directly influence the structural integrity of the vessel wall and underlying basement membranes through excessive crosslinking of matrix molecules such as collagen and disruption of matrix-matrix and matrix-cell interactions (32–33). Intracellularly, nonenzymatic glycation of intracellular molecules such as basic fibroblast growth factor may lead to impaired function of this molecule (34). In the vasculature, AGE have been shown to quench the action of nitric oxide (NO), thereby adversely affecting vascular relaxation and function (35). The impaired ability of diabetic vasculature to respond to stimuli such as acetylcholine in human subjects as well as in experimental models has suggested that endothelial dysfunction may be a predictor of diffuse vascular disease and atherosclerosis (36–37). Suggestive evidence for a role for
AGE in modulating gene expression linked to diabetic glomerular disease was derived from the studies suggesting that AGE enhanced expression of growth factors linked to extracellular matrix proteins by mesangium (38). Further, incubation of cultured human retinal pigment epithelium and bovine vascular smooth muscle cells with AGE was shown to increase transcripts for VEGF (39). In vivo, Yang et al. (40) showed that infusion of AGE-modified murine serum albumin into nondiabetic mice for 4 wk caused upregulation of glomerular alpha 1 (IV) collagen, laminin B1, and TGF-β1 transcription in the kidney, in parallel with glomerular hypertrophy.

On the basis of these findings and other evidence suggesting roles for AGE in the pathogenesis of diabetic complications, pharmacologic inhibitors of AGE formation were developed to test the concept that reduced AGE formation would lead to decreased complications. The best example of these is aminoguanidine (AG), an agent of which the major biologic target is AGE formation. It was discovered, however, that the agent possessed additional properties, such as suppression of NO formation by inhibition of inducible nitric oxide synthase, thereby creating a potentially paradoxical situation whereby, depending on the site and specific biologic context, AG might exert beneficial and/or adverse effects (41).

When tested in vivo in experimental animal models, a plethora of evidence emerged that AG reduced complications in a number of organ systems in diabetic animals. In the kidney, administration of AG to diabetic rats prevented the development of albuminuria (42–43) in parallel with normalization of glomerular PKC (44). More recently, this concept was tested in nonhuman primates; in a baboon model of type 1 diabetes, administration of AG significantly reduced the thickening of the glomerular basement membrane. However, diabetic animals receiving AG still developed albuminuria. Furthermore, even nondiabetic AG-treated animals developed albuminuria (45).

Ultimately, when tested in humans, a clinical trial of AG in diabetic subjects with nephropathy demonstrated a trend toward improving renal function (46–47). Indeed, on the basis of data in animals and promising studies in human subjects, evidence supports the concept that reduction of AGE burden favorably affects the course of diabetic complications. In this context, it is important to note that other agents that act as inhibitors of AGE formation, such as ALT-946, OPB-198, and pyridoxamine have been developed and are in multiple stages of development. These agents have shown efficacy in multiple experimental settings as they reduce a range of diabetes-associated complications in animal models (48–50).

In addition to inhibitors of AGE formation, parallel efforts have focussed on development of novel agents to breakdown preformed AGE. In testing in cardiovascular disease, one such agent, ALT-711, was found to reverse age-related myocardial stiffness and improve arterial compliance (51–53). Issues to be addressed using these agents, however, include identification of their precise biochemical targets, as well as the fate of the moieties released upon action of the drug on preformed AGE.

Taken together, the use of agents such as AG and AGE crosslink breakers has provided support for the premise that AGE pathways contribute to the pathogenesis of diabetes-associated renal dysfunction. Furthermore, identification of the ability of these molecules to engage cell surface receptors and initiate changes in gene expression and cellular function has led to a new potential target for therapeutic intervention in diabetic complications.

**Receptor-Dependent Pathways.** In addition to targeting formation/stability of AGE in clinical intervention, the concept that AGE take on a “gain of function” in the diabetic tissues is linked to the observation that AGE-modified proteins bind vascular and inflammatory cells by interaction with specific cell surface molecules. Such cell surface interaction sites include molecules such as the macrophage scavenger receptor (MSR) type II, OST-48, 80K-H, galectin-3, CD36, and receptor for AGE (RAGE) (54–59).

In contrast to receptors of which their role is to detoxify/remove AGE from the circulation/tissues, RAGE is a signal transduction receptor for AGE of the immunoglobulin superfamily that mediates diverse cellular responses. Engagement of RAGE amplifies the development of complications, particularly in a ligand-enriched environment.

**RAGE: A Multi-Ligand Receptor of the Immunoglobulin Superfamily**

Consistent with the premise that receptors for AGE would gain the potential to bind selectively AGE-modified molecules, RAGE was first identified as a “receptor” for AGE using bovine lung extract and a [125I]-radiolabelled probe of in vitro-prepared AGE albumin (58). In cultured cells, such as endothelial cells (EC) and mononuclear phagocytes (MP), AGE bound in a dose-dependent and saturable manner, largely via engagement of RAGE, as evidenced by blockade of binding by either anti-RAGE IgG or soluble (s) RAGE, the extracellular ligand-binding domain of RAGE (58–63). Molecular cloning analyses revealed that RAGE was a member of the immunoglobulin superfamily of cell surface molecules (59). The predicted hydropathy plot for bovine RAGE indicated that the extracellular portion of the molecule was composed of 332 amino acids and consisted of one V-type immunoglobulin domain, followed by two C-type immunoglobulin domains. A series of studies on truncations of the extracellular region demonstrated that its ligands interacted with the V-domain of the receptor (63–64). Following the extracellular region, there is a single hydrophobic transmembrane spanning domain; this portion of the molecule is followed by a short, highly charged cytosolic tail at the carboxyl terminus. Our studies have shown that this intracellular portion of the molecule is essential for RAGE-triggered signaling, as deletion of the cytosolic domain imparts a dominant negative effect, in vitro and in vivo (59, 64).

Interestingly, not unlike other members of the immunoglobulin superfamily, RAGE engages distinct ligands beyond AGE. In this context, we recently identified that a specific class of AGE, CML-modified adducts, also serves as a signal transduction ligands for RAGE (60). In addition, RAGE interacts with amyloid-beta peptide and beta-sheet fibrils that form in settings such as amyloidoses (65–67). RAGE also interacts with amphoterin, a member of the HMG (high mobility group)-1 family of DNA-binding proteins that exist both intracellularly and on the surface of cells, such as embryonic neurons and tumor cells, and have
been linked to cellular migration and invasion potential (68–69). Further, RAGE is a signal transduction receptor for at least certain members of the family of S100/calgranulins (64). S100/calgranulins, members of a large family comprising over 15 molecules, many with proinflammatory potential, have central functions within the cell, where their roles are linked to homeostatic properties, such as calcium binding, as well as distinct properties outside the cell. S100/calgranulins exist extracellularly in multiple contexts, particularly in proinflammatory milieu (70–72). In the latter context, the biology of S100/calgranulins has led to new and exciting hypotheses about the role of RAGE in pathologic settings. In *vitro*, we found that a prototypic S100/calgranulin, S100A12, activated EC, MP, and peripheral blood mononuclear cells (PBMC) in a manner linked to generation of cytokines and adhesion molecules (64). Consistent with a potential role in proinflammatory processes *in vivo*, blockade of RAGE in euglycemic mice suppressed delayed type hypersensitivity induced by sensitization/challenge with methylated BSA; diminished colonic inflammation in mice deficient in IL-10; and decreased phenotypic and molecular indices of arthritis in dba/1 mice subjected to sensitization/challenge with bovine type II collagen (64, 73). Here, RAGE was found not to be the initiating cause of the immune/inflammatory event, but rather a key component of an amplification pathway leading to sustained inflammation and tissue injury.

On the basis of these considerations, we hypothesized that in diabetes, triggers of RAGE activation likely included multiple molecular species. Consistent with this premise, for example, we found that diabetic atherosclerotic lesions in apo E–deficient mice were enriched not only in AGE, but also in S100/calgranulins (74). In so far as the complications of diabetes can be viewed as an exaggerated inflammatory response, it is highly plausible that in diabetic tissues, S100/calgranulins are likely not “innocent bystanders,” but, rather, contributing factors to the biology of accelerated tissue perturbation that typifies the development of complications.

These considerations led us to test the impact of RAGE in key settings perturbed in diabetic tissues, such as the large blood vessels and the kidney.

**RAGE: Tissue Localization and Upregulation in Disease States**

A key focus of our efforts to dissect the role of RAGE in distinct pathologic settings was to identify the specific cell types in each pathologic context expressing the receptor. In homeostasis in humans and in animals, RAGE is present at low levels in the adult (75–76). RAGE mRNA and antigen are found in a wide range of cell types, including endothelial cells (EC), mononuclear phagocytes (MP), lymphocytes, vascular smooth muscle (VSMC), and neurons. Interestingly, in the kidney, despite the striking impact of RAGE in MP or SMC biology, RAGE was selectively expressed in the glomerular visceral epithelial cells (podocytes), and not the mesangium or glomerular endothelium (77). During embryonic development, the expression of RAGE is increased in neurons of the central nervous system, including those within the cerebral cortex, cerebellum, and hippocampus. Although these findings initially suggested that genetic deletion of RAGE might result in embryonic lethality or in severe impairment of growth/cellular functions, especially within the brain, the generation of homozygous RAGE null mice has not supported this premise (78). RAGE null mice are viable, display normal lifespan and fertility, and appear to exhibit phenotypic differences compared with wild-type animals selectively upon induction of certain stresses, such as femoral artery denudation injury and induction of diabetes (79). Thus, upon induction of diabetes or other forms of stress, these animals provide a valuable template for the dissection of the role of RAGE-mediated processes in the pathogenesis of diabetic complications.

**Blockade of RAGE: A Target for the Treatment of Diabetic Complications in the Vasculature**

The striking upregulation of RAGE and its ligands, AGE and S100/calgranulins, in diabetic blood vessels in human subjects and murine models (80–81) suggested that interruption of this axis might favorably affect the initiation and/or progression of complications in the vasculature.

![Figure 1](https://example.com/figure1.png)

*Figure 1.* Receptor for advanced glycation endproducts (RAGE) is expressed in podocytes in human kidney. Sections were prepared from human control (nondiabetic) and diabetic kidney and immunostaining performed using anti-human RAGE IgG (a and b) or anti-synaptopodin IgG (c). Scale bar: 25 μm in a; 15 μm in b and c.
RAGE and Diabetic Vascular Hyperpermeability

As a first test of the involvement of the ligand-RAGE axis in diabetic vascular perturbation, the effect of RAGE on barrier function of diabetic vasculature was studied. Increased vascular leakage is a well-known feature of diabetic microvasculature linked to the development of gross disease in vascular structures (82–83). To study this, we employed rodent models of hyperglycemia. Diabetes was induced in rats using the
β-cell toxin streptozotocin. By 11 wk after administration of streptozotocin, diabetic animals displayed increased vascular leakage, as demonstrated by the tissue-blood isotope ratio (TBIR) (84). Increased vascular permeability in diabetic rats was most evident in intestine, skin, and kidney, where albumin leakage was increased significantly compared with nondiabetic controls (84). To block the ligand-RAGE interaction, diabetic rats were treated with a single dose of sRAGE, 2.25 or 5.15 mg/kg. Administration of sRAGE at the lower dose completely blocked vascular leakage in intestine and skin, and largely prevented it in the kidney (~60%). In the presence of the higher dose of sRAGE, hyperpermeability was completely suppressed in intestine and skin and by ~90% in kidney (84). These findings were the first demonstration in vivo that blockade of RAGE might suppress a dynamic complication of the diabetic state. We extended these concepts to accelerated macrovascular disease in diabetes to establish the relationship between RAGE blockade and modulation of vascular disease. In diabetic apo E null mice, blockade of RAGE potently suppressed early accelerated atherogenesis and the progression of established lesions (81, 85–86).

Examination of the lipid and glycemic profile revealed that RAGE blockade did not exert its beneficial effects by modulation of these pathways. In addition, an unexpected observation was the finding that levels of AGE were reduced in sRAGE-treated diabetic animals (81). These findings raise the likely possibility that RAGE contributes to ongoing generation of oxidant stress and, thereby, sustained production of reactive oxygen species. Once set in motion, this cascade of events proceeds to accelerated vascular injury unless interrupted. Consistent with this premise, we observed diminished susceptibility of LDL to copper-induced oxidation in LDL retrieved

Figure 3. Expression of RAGE antigen, CML/AGE, and S100/calgranulin is enhanced in db/db kidney. Sections were prepared from nondiabetic m/db mice (a, c, and e) or db/db mice (b, d, and f) at age 13 wk and immunostaining performed using anti-RAGE IgG (a and b), anti–CML IgG (c and d), or anti–S100 IgG (e and f). Scale bar: 16 μm.
been shown that there are increased numbers of mononuclear cell recruitment/attraction of MPs (88–89). Interestingly, it has been shown that the podocyte is the principal VEGF-expressing cell in the diabetic kidney. Two specific functions of VEGF suggested its probable link to diabetic glomerular disease; VEGF mediates hyperpermeability and, in addition, recruitment/attraction of MPs (88–89). Interestingly, it has been shown that there are increased numbers of mononuclear inflammatory cells in the glomeruli in early experimental models of diabetes, as well as in human diabetic kidney (90–91).

These considerations led us to hypothesize that increased accumulation of AGE and S100/calgranulins engaging podocyte RAGE in the diabetic glomerulus upregulates expression of VEGF, leading to increased permeability and enhanced attraction of proinflammatory mononuclear cells, thereby setting the stage for glomerular albuminuria as well as an inflammatory process likely to promote mesangial activation and, ultimately, expansion. To address the impact of RAGE in our first studies, we administered murine sRAGE to db/db mice from age 8 wk to age 13 or 27 wk. In db/db mice treated with sRAGE from age 8 to 13 wk, increased VEGF antigen expression at age 13 wk was prevented (78). In parallel, decreased numbers of mononuclear inflammatory cells were evident in the glomeruli of sRAGE-treated db/db mice (78).

These observations at an early stage of diabetes led us to assess the impact of long-term blockade of the receptor in diabetic mice. In renal cortical tissue retrieved from sRAGE-treated db/db mice, Northern blotting revealed decreased transcripts for TGF-β compared with vehicle-treated db/db mice (Figure 4), thus suggesting that mesangial expansion might be prevented/delayed in the presence of blockade of the receptor. Periodic acid-Schiff–staining of renal cortical tissue retrieved from db/db mice treated with sRAGE revealed decreased glomerular and mesangial area compared with vehicle treatment (Figure 5). Further, PBS-treated db/db mice displayed increased thickness of the GBM compared with nondiabetic

**RAGE and Diabetic Nephropathy**

**Studies in Human Diabetic Nephropathy.** Whereas low-level RAGE expression was restricted to podocytes in normal control human glomeruli, glomeruli of patients with diabetic nephropathy demonstrated diffuse upregulation of RAGE expression in podocytes, co-localizing with synaptopodin expression (77) (Figure 1). No expression of CML-AGE could be detected in normal human glomeruli (Figure 2a). In both diffuse and nodular diabetic nephropathy, CML was identified by immunohistochemistry in mesangial matrix, glomerular basement membranes, tubular basement membranes, and many vessel walls (Figure 2, b through e). The intensity of CML immunostaining was greatest in advanced diabetic nephropathy with extensive glomerular sclerosis (Figure 2f). Staining for S100/calgranulin could be identified in the distribution of infiltrating MP in the glomeruli of diabetic nephropathy (Figure 2g).

**Studies in db/db Mice.** The observation that ligands for RAGE and the receptor itself were upregulated in human diabetic kidney prompted us to address the role of this axis in diabetic kidney disease. To address these concepts experimentally, we first turned to the db/db mouse, a model of sustained hyperglycemia driven by insulin resistance in which the changes associated with long-term diabetes parallel, at least in part, those evident in human diabetic kidney (87). Consistent with observations in the human kidney, immunohistochemistry revealed that the principal site of RAGE expression in the glomerulus of the db/db mouse was the podocyte, and that immunoreactivity was enhanced compared with nondiabetic controls (Figures 3b and 3a, respectively). To confirm that the principal site of RAGE expression was the podocyte, we performed immunohistochemistry using anti-synaptopodin IgG. These studies revealed that RAGE expression overlapped with expression of synaptopodin. Consistent with multiple studies in human subjects, CML-AGE adducts were upregulated in the kidney of db/db mice versus control animals (Figures 3d and 3c, respectively), as were S100/calgranulin antigens (Figures 3f and 3e, respectively). S100/calgranulin-expressing cells were demonstrated to be mononuclear phagocytes based on colocalization experiments using anti-Mac-3 IgG (78).

These data suggested that RAGE and its ligands were enriched in the diabetic glomerulus, especially in podocytes. The particular localization of RAGE in the podocyte led us to explore the potential contribution of VEGF in this setting, as it is well-established that the podocyte is the principal VEGF-expressing cell in the diabetic kidney. Two specific functions of VEGF suggested its probable link to diabetic glomerular disease; VEGF mediates hyperpermeability and, in addition, recruitment/attraction of MPs (88–89). Interestingly, it has been shown that there are increased numbers of mononuclear

![Figure 4. Increased transcripts for TGF-β1 in db/db kidney: suppression by blockade of RAGE. db/db mice were treated with murine sRAGE or vehicle, PBS, from age 8 through 27 wk. At age 27 wk, renal cortical tissue was retrieved from the indicated mice and Northern blotting using labeled probes to either TGF-β1 or β-actin performed. ** P < 0.01.](image-url)
controls, and this was largely prevented in the presence of blockade of RAGE (78).

In parallel with significant improvement in structural properties of the diabetic glomerulus, the key test of these concepts was the impact on function. We found that at age 27 wk, increased urinary albumin excretion in db/db mice was largely prevented in the presence of sRAGE (Figure 6).

Taken together, these observations strongly suggested that pharmacologic blockade of RAGE suppressed structural, functional, and molecular components associated with diabetes-associated nephropathy. To study this in mice with relative insulin deficiency and in mice bearing genetic modification of RAGE, we studied homozygous RAGE null mice.

**Studies in RAGE Null Mice.** Homozygous RAGE null mice are viable and display normal lifespan and may thus be employed to address the role of RAGE in diabetes-associated complications. In these mice and wild-type littermates, we employed streptozotocin to induce hyperglycemia secondary to relative insulin deficiency and examined the affect of deletion of RAGE on the course of early diabetes in the kidney. After 6 wk of diabetes, although diabetic wild-type littermates displayed increased VEGF antigen in the renal cortex compared with nondiabetic littermates, diabetic RAGE null mice did not demonstrate increased VEGF antigen compared with nondiabetic RAGE null mice (Figure 7). Similarly, Northern blotting revealed that transcripts for TGF-β were not increased in diabetic versus nondiabetic RAGE null mice; in contrast, wild-type littermates with diabetes displayed increased transcripts for TGF-β compared with wild-type littermate animals (78).

Indeed, in these first studies in early diabetes, although diabetic wild-type mice displayed significantly increased mesangial matrix and thickening of the glomerular basement membrane, RAGE null mice with diabetes failed to display these differences (73). Studies are ongoing to extend these experiments to longer time points to assess the impact of RAGE deletion on renal function in diabetic animals. Importantly, in both settings, db/db mice and RAGE null animals, blockade/deletion of RAGE does not affect levels of blood glucose, body weight, or lipid profile.

These observations have formed the basis for the following premise. We hypothesize that in hyperglycemia, accelerated production of AGE and release of S100/calgranulins, in part from inflammatory cells infiltrating the glomerulus stimulated directly by high glucose and by AGE, upregulates expression of podocyte RAGE. A key consequence of upregulated RAGE is increased expression of VEGF, which exerts potent effects,
on both hyperpermeability and the development of albuminuria, and the incitement of further migration and activation of inflammatory MP into the glomerulus. Such MP release a broad array of molecular species that enhance generation of TGF-β and other matrix proteins, a process leading to mesangial activation and expansion, and thickening of the glomerular basement membrane. Taken together, these processes culminate in glomerular sclerosis. Further, an amplification loop within this cascade is set in motion; activated MP are a rich source of S100/calgranulins, thereby triggering further upregulation of RAGE, cellular perturbation and glomerular dysfunction.

Much certainly remains to be done to rigorously test these concepts experimentally. Specifically, to address the distinct contribution of RAGE in MP and podocytes to the development of diabetic kidney disease, we have prepared targeted transgenic mice bearing signaling-deficient mutants of RAGE in either MP or podocytes, driven by specific promoters. Our goal is to render these animals diabetic and assess the effect on glomerular inflammation, structure, and function. Using these animals, the temporal and site-specific contribution of RAGE signaling in these two key RAGE-bearing cell types may be best addressed.

In this context, it is essential to point out that in previous studies, Yamamoto et al. (92) showed that targeted overexpression of RAGE in endothelial cells, and to some degree cells of MP lineage, accelerated processes linked to diabetes-associated nephropathy in mice. These considerations underscore the possibility that endothelial expression of RAGE, although not readily detectable in our studies, also contributed to perturbation in the diabetic kidney. However, the demonstration that monocytes also expressed the RAGE transgene does not definitively exclude an important role of MP RAGE in the observed changes in the diabetic kidney in that experimental model (92).

It must also be noted that tubulointerstitial processes contribute importantly to the pathogenesis of diabetes-associated nephropathy. In the context of RAGE, Oldfield et al. (93) showed that AGE-RAGE interaction in cultured renal proximal tubular epithelial cells resulted in epithelial-myofibroblast transdifferentiation. Although limited mechanistically to the in vitro setting, these fascinating observations suggest that the interplay of glomerular podocyte RAGE and tubular RAGE may contribute to the sum of alterations that ensue in the diabetic kidney. Such considerations provide an exciting area for further investigation in the study of RAGE biology in the diabetic kidney.

**RAGE and Other Glomerular Diseases**

Lastly, these data in diabetic kidney disease have highlighted an important role for RAGE in an amplification pathway leading to progressive structural/functional impairment in the kidney. The observation that the principal RAGE-bearing cell in the glomerulus is the podocyte has additional intriguing implications that we have recently begun to address experimentally. Prompted by the observation that central roles for podocytes have been linked to other forms of nephropathy, especially those linked to oxidative bursts, such as experimentally-induced passive Heymann nephritis (PHN), we hypothesized that adriamycin-induced generation of reactive oxygen species in the glomerulus might ensue, in part, secondary to RAGE-dependent processes. Our previous studies demonstrated that engagement of RAGE activates NADPH oxidase species in endothelial cells and MP, thereby triggering signal transduction mechanisms such as p21ras, erk 1/2 MAP kinases, and NF-κB in an oxidant-sensitive manner (94–96). It was thus plausible that adriamycin-induced oxidant stress might drive activation of podocyte RAGE, and, therefore, set the stage for cellular perturbation. Consistent with these hypotheses, in BALB/c mice treated with adriamycin, blockade of RAGE
Conclusions and Perspectives

Our studies to date strongly suggest that activation of RAGE prompts an amplification mechanism in settings such as diabetes and inflammatory foci whereby accelerated ligand accumulation sets up a vicious cycle of cellular activation. Unless interrupted, irremovable injury ensues, coupled with failure of reparative mechanisms. As homozygous RAGE null mice appear free of an overt phenotype in the absence of induced stresses, these considerations have led us to consider if endogenous properties of the receptor contribute importantly to beneficial host responses. In this context, an ideal setting in which to address these issues is in acute peripheral nerve injury. In that milieu, a sharply limited inflammatory response, encompassed within the process of Wallerian degeneration, in concert with peripheral axonal outgrowth are essential for recovery of function and regeneration. As RAGE participates in both inflammatory mechanisms and neurite outgrowth, we tested the effect of blockade of RAGE on unilateral sciatic nerve crush in a murine model. Both sRAGE and administration of blocking F(ab')<sub>2</sub> fragments prepared from anti-RAGE IgG suppressed neuronal functional and structural regeneration (98). These considerations highlight a “bright side” in the biology of RAGE and underscore the notion that such a receptor of the immunoglobulin superfamily most surely participates in innate host responses to injury, thus bestowing and augmenting regenerative potential in at least certain examples of acute stress.

Thus, the potential for future therapeutic intervention strategies targeting RAGE will emerge upon rigorously probing the endogenous roles of this receptor in acute or chronic host perturbation. The viability of the RAGE null mouse holds promise for the future studies of RAGE blockade in disorders of the kidney and beyond.

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