Development of age-dependent glomerular lesions in galectin-3/AGE-receptor-3 knockout mice

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ABSTRACT

To investigate the role of the AGE/AGE receptor pathway in the pathogenesis of age-related renal disease, we evaluated the development of glomerular lesions in aging galectin-3 knockout (KO) vs. wild-type (WT) mice and their relation to the increased AGE levels and oxidative stress characterizing the aging process. KO mice showed significantly more pronounced age-dependent increases in proteinuria, albuminuria, glomerular sclerosis, and glomerular and mesangial areas, starting at 18 mo, as well as renal extracellular matrix mRNA and protein expression, starting at 12 mo vs. age-matched WT mice. Circulating and renal AGEs, plasma isoprostane 8-epi-PGF2α levels, glomerular content of the glycoxidation and lipoxidation products Nε-carboxymethyllysine and 4-hydroxy-2-nonenal, and renal nuclear factor-κB activity also increased more markedly with age in KO than WT mice. AGE levels correlated significantly with renal functional and structural parameters. These data indicate that aging galectin-3 KO mice develop more pronounced changes in renal function and structure than coeval WT mice, in parallel with a more marked degree of AGE accumulation, oxidative stress, and associated low-grade inflammation, thus supporting the concept that the AGE/AGE receptor pathway is implicated in age-related renal disease.

age-related glomerulopathy; advanced glycation end-products; advanced glycation end-product receptors; oxidative stress

AGING IS A COMPLEX PATHOPHYSIOLOGICAL condition in which the function of many organ systems becomes altered, although it is unclear the extent to which these changes are the result of a normal aging process or of the interplay of age with chronic diseases that are more common in older people. Several changes in kidney function and structure have been detected in aged humans (31) and animals (4). The functional hallmark is a progressive decline of glomerular filtration rate, associated with reduced renal plasma flow and ultrafiltration coefficient, and increased filtration fraction and renal vascular resistance (19, 31). From a morphological point of view, the glomerular number is reduced (36), and there is an increased prevalence of global glomerulosclerosis and tubulointerstitial fibrosis, preceded and accompanied by glomerular hypertrophy, mesangial matrix expansion, glomerular basement membrane thickening, and arteriolar hyalinosis (4, 31). These structural changes resemble those detected in diabetes (18).

Also, aging shares with diabetes some biochemical abnormalities that might play a major role in the pathogenesis of vascular and renal changes occurring in both conditions. One popular theory proposes that the characteristic loss of elasticity of tissues of aged individuals is dependent on the progressive accumulation of advanced glycation end-products (AGEs; see Ref. 49). An AGE cross-link breaker was shown to reverse age-related increases in myocardial and arterial stiffness (2, 54). The aging process has also been related to cumulative oxidative damage caused by reactive oxygen species (ROS) over a lifetime (28). In both invertebrates and mammals, increased longevity correlates with enhanced resistance to oxidative stress (12), and deletion of p66Shc, a protein involved in ROS-induced apoptosis, was shown to be associated with increased resistance to oxidative stress and increased life span (35).

These biochemical abnormalities are strictly related to each other. In fact, AGEs are heterogeneous compounds that accumulate in tissues due to increased formation/intake and reduced degradation/excretion; AGE formation occurs through both nonoxidative and oxidative reactions (11). Enhanced mitochondrial superoxide production, resulting from excess glucose disposal and causing diversion of glucose flux toward the AGE-precursor methylglyoxal via inhibition of glyceraldehyde phosphate dehydrogenase, is now considered as the main mechanism of AGE formation in diabetes (7). Aging is also characterized by less efficient electron transport in the mitochondrial respiratory chain, with consequent increase in superoxide production, and calorie restriction, an intervention known to prolong life span, was found to proportionately decrease mitochondrial ROS generation, especially at complex I (3, 50). AGEs exert both direct (physicochemical) and indirect (biological) effects, the latter mediated by cell surface receptors. AGE receptors have a dual function, since they are involved in AGE removal, and also in AGE-induced cell apoptosis, was shown to be associated with increased resistance to oxidative stress and increased life span (35).

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nuclear factor (NF)-κB (6, 32) and the modulation of gene expression of several cytokines, with consequent induction of a state of chronic low-grade inflammation that characterizes both diabetes and aging and results in coagulation, vasoconstriction, adhesion, and, particularly, altered cell and extracellular matrix (ECM) turnover (47).

The numerous AGE-binding proteins include the receptor for AGEs (RAGE; see Ref. 47), mainly involved in cell activation, the macrophage scavenger receptors (MSRs) class A (20) and B (38), which participate in AGE removal, and OST-48/AGE-receptor (R) 1 (33), 80K-H/AGE-R2 (33), and galectin-3/AGE-R3 (51), thought to behave as an AGE receptor complex.

Galectin-3 consists of a short NH2-terminal domain continuing into a unique proline-glycine-alanine-thyrosine-rich repeat motif and a highly conserved COOH-terminus containing the carbohydrate recognition domain (CRD; see Ref. 41). These unique structural properties and the dual localization of galectin-3 determine two different modes of interaction with proteins (via its NH2-terminus and CRD, respectively) and enable it to exert multiple functions (40). Intracellularly, galectin-3 acts as a pre-mRNA splicing factor (10) and also regulates the cell cycle, with promotion of replication and inhibition of apoptosis (58). Extracellularly, galectin-3 regulates cell adhesion in a dual manner: it promotes homo- and heterotypic cell-to-cell interactions (24), whereas it downregulates cell adhesion to the ECM (37). Extracellular galectin-3 is also capable of interaction with IgE and the IgE receptor, which induces mast cell activation (13) and the high-affinity binding, internalization, and degradation of AGEs (51, 60).

Recently, we have reported that galectin-3 is not or is weakly expressed at the glomerular/mesangial level under normal conditions and is induced/upregulated in diabetes (43) and that galectin-3 knockout (KO) mice are more prone to develop glomerular disease induced by diabetes and AGE injection compared with the corresponding wild-type (WT) animals (23, 42). The more marked increases in circulating and renal tissue AGE levels in the diabetic (and AGE-injected) KO mice vs. WT mice and the altered AGE receptor expression pattern in the galectin-3-deficient genotype suggested that it was attributable to the lack of galectin-3 AGE receptor function, thus providing evidence that galectin-3 plays a significant role in vivo to afford protection toward AGE-induced tissue injury, as opposed to RAGE (22).

This study was aimed at investigating the role of the AGE/AGE receptor pathway in the pathogenesis of age-related renal disease by assessing the development of glomerular lesions in galectin-3 KO mice and their relation to the increased AGE levels and oxidative stress characterizing the aging process. The working hypothesis was that galectin-3 KO mice develop accelerated age-related renal disease compared with coeval WT mice because of their increased susceptibility to AGE-induced injury.

METHODS

Experimental design. Male galectin-3 KO mice, generated by gene ablation (21), and the corresponding C57BL6 WT mice were studied at 2, 6, 12, 18, and 24 mo of age (n = 6–8 animals/group). The animals were housed and cared for in accordance with the Principles of Laboratory Animal Care (NIH Publication no. 85–23, revised 1985) and national laws and had free access to food and water. Before death, mice were placed in metabolic cages to collect urine samples for total protein, albumin, and creatinine measurements. Next, the animals were weighed and anesthetized with intraperitoneal ketamine (Fmalgene, 60 mg/kg body wt) and xylazine (Rompun, 7.5 mg/kg body wt), a laparotomy was performed, and a blood sample was obtained for measurement of blood glucose, serum creatinine and AGE levels, and plasma isoprostane 8-epi-prostaglandin PGF2α concentrations. Finally, both kidneys were quickly removed, cleaned of surrounding fat, washed in sterile saline solution, and weighed. A sagittal section of the right kidney was immediately fixed by immersion in phosphate-buffered 4% formaldehyde solution and processed for light microscopic examination and morphometric evaluation, as previously described (23, 42). Paraffin-embedded sections were used also for immunohistochemistry for fibronectin and collagen IV, to assess the extent of renal ECM accumulation, as well as Nα-carboxymethyllysine (CML) and 4-hydroxy-2-nonenal (HNE), to assess the extent of glycoxidation and lipoxidation reactions, respectively (5), and the distribution of these products within the kidney tissue. The remaining tissue from the right kidney was frozen in liquid nitrogen and subsequently used for either total or nuclear protein extraction, as previously described (23). Total protein extracts were used for the assessment of fibronectin and collagen IV protein expression and AGE content, whereas nuclear extracts were used for measurement of NF-κB activity. The renal cortex from the left kidney was separated from medulla and used for total RNA extraction by the guanidine thiocyanate-phenol-chloroform method using TRIzol Reagent (Invitrogen Italia, San Giuliano Milanese, Italy). The kidney cortex mRNA levels of the following targets were then assessed: the ECM components fibronectin, laminin B1, and collagen IV α1-chain; the prosclerotic cytokine transforming growth factor (TGF)-β1; and the AGE receptors OST-48/AGE-R1, 80K-H/AGE-R2, galectin-3/AGE-R3, RAGE, and MSR-A type II.

Renal function. Serum creatinine levels were measured by the Jaffé method (23, 42). Total proteinuria was assessed by the Bradford method using a Bradford dye-binding protein assay kit (Pierce Chemical, Rockford, IL), whereas albuminuria was measured with a Mouse Albumin ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, TX); both values were normalized by urine creatinine concentration (ng/μg; see Refs. 23 and 42).

Renal structure. Analysis of renal structure was performed by two pathologists blinded to the group assignment of the specimens on multiple 4-μm kidney tissue sections stained with periodic acid-Schiff (PAS). Sections were evaluated for glomerular sclerosis, as assessed by a standard semiquantitative analysis of 100 glomeruli/animal and expressed as glomerular sclerosis index (GSI; see Ref. 23). One hundred glomeruli per animal were graded as 0, 1, 2, 3, or 4, according to absent, <25, 25–50, 51–75, or >75% cross-sectional sclerosis, respectively. The GSI for each mouse was calculated by the formula: (N1 x 1 + N2 x 2 + N3 x 3 + N4 x 4)/n, where N1, N2, N3, and N4 represent the numbers of glomeruli exhibiting grades 1, 2, 3, and 4, respectively, and n is the total number of glomeruli assessed (i.e., 100). Morphometric analysis was performed by the use of a custom-made, C-language macro written with the Optimas 6.5 image analysis system (Optimas; MediaCybernetics, Silver Spring, MD), as previously reported in detail (23). Briefly, the areas of at least 60 glomerular tuft profiles/sample were measured, the harmonic mean of the profile area (mean glomerular area, mGA) was obtained, and the mean glomerular volume (mGV) was estimated from it. Next, periodic acid-Schiff (PAS)-positive material in each of these glomeruli was quantified and expressed as the percentage of the glomerular tuft area (fractional mesangial area, fMA). The color threshold was set by identifying three to five separate pixels in areas of positive staining. Finally, the mean mesangial area (mMA) was calculated by the formula (fMA x mGA)/100.

Renal ECM, TGF-β1 and AGE receptor gene expression. Transcripts for fibronectin, laminin B1, collagen IV α1-chain, TGF-β1, AGE-R1, AGE-R2, galectin-3/AGE-R3, RAGE, and MSR-A type II
were quantified by competitive RT-PCR (23, 42). Total RNA (1 μg) was reverse transcribed using a Retroscript kit (Ambion, Austin, TX). The following primers were used: fibronectin sense 5'-AGC GGT GTC TAC TCT GT-3' and antisense 5'-GAT GCA GAT ATC TCG GAG ATC-3'; laminin B1 sense 5'-CAA GCT TGA CCA TAG TGG TCC-3'; collagen IV α1-chain sense 5'-TAG GTG TCA GCA ATT AGG CAG G-3' and antisense 5'-TGA CCT CCA CTC CCT ACT CCT-3' and antisense 5'-GTC GAC AGG CAA CTA AAT ATA GG-3'; AGE-R1 sense 5'-GCT CTT CCA CTC CTT ACT CCT-3' and antisense 5'-CCC GAC AGG CAA CTA TGA AC-3'; AGE-R2 sense 5'-TGG TGT GGT GGC TTT CGA TTC AGC-3'; AGE-R3/galectin-3 sense 5'-CAC CTG CAC CTG GAG TCT AC-3' and antisense 5'-GCA GTG TGT AGG TCT ATG TC-3'; laminin B1 sense 5'-GGT GTC TAC TCT GT-3' and antisense 5'-CCA GCT TGA GAG AGG GTG-3'; and antisense 5'-CCA GAG CAA CTA TGA AC-3'; AGE-R3/galectin-3 sense 5'-CAC CTG CAC CTG GAG TCT AC-3' and antisense 5'-GCA GTG TGT AGG TCT ATG TC-3'; RAGE sense 5'-CCT GGG AGG CAA GAA ATT-3' and antisense 5'-GCC CAG CTC GAG TCT AC-3'.

Plasma isoprostane 8-epi-PGF2α levels. Plasma levels of isoprostane 8-epi-PGF2α, an index of systemic oxidative stress, were determined by ELISA using a commercial kit (Cayman, Ann Arbor, MI).

CML and HNE content. Renal content of CML and HNE was assessed in paraffin-embedded sections by immunohistochemistry (23). After deparaffinization and antigen retrieval with 0.1% trypsin (Dako) for 30 min at 37°C, endogenous peroxidase reactivity and nonspecific binding sites were blocked as described above. Sections were incubated overnight at 4°C with a biotinylated mouse monoclonal antibody against CML (Clone No. 1F-1G; Wako Chemicals, Neuss, Germany) at a concentration of 3 μg/ml or a rabbit antisem against HNE (Alpha Diagnostic International, San Antonio, TX), diluted 1:1,000 in 10 mM PBS containing 1% normal goat serum, followed (for HNE detection only) by a biotinylated goat anti-rabbit IgG antibody, diluted 1:500, for 1 h at room temperature. After incubation with StrepABCComplex/HRP (Dako) for 20 min, samples were developed and counterstained as described above. In the negative controls, the primary antibody was omitted or replaced with nonimmune serum. Results were expressed as percent glomerular area that was positive to each product.

Renal NF-κB activity. The activity of NF-κB p65 was assessed by ELISA using a TransAMNF-κB p65 Transcription Factor Assay Kit (Active Motif, Carlsbad, CA; see Ref. 23). This method is based upon reaction of nuclear extracts (30 μg protein) with specific oligonucleotide sequences coated on a microtiter plate. According to the manufacturer’s instructions, the binding of activated NF-κB was revealed by the addition of a primary polyclonal anti-NF-κB p65 antibody, followed by a secondary antibody conjugated with horseradish peroxidase and the 3,3',5,5'-tetramethylbenzidine substrate. Absorbance was finally read at 450 nm and, after the reaction was stopped with sulfuric acid, 655 nm using a microtiter plate reader.

Statistical analysis. Values are expressed as means ± SD; the percent change in KO vs. WT animals was also calculated. Statistical significance was evaluated by Student’s t-test for comparison between KO and WT mice at each age and one-way ANOVA for comparison among different age groups. Correlation between serum or kidney tissue AGE levels and parameters of renal function and structure were also calculated using linear regression analysis. All statistical tests were performed on raw data.

RESULTS

Body weights. Significant body growth occurred only from 2 to 6 mo of age and, to a lesser extent, from 6 to 12 mo. Thereafter, body weight remained stable, with no significant difference between the two genotypes at any age (Table 1).

Renal function. Serum creatinine levels increased only slightly (by ∼10%) from 2 to 24 mo of age, with no significant difference between KO and WT mice (Table 1). Urinary protein/creatinine and albumin/creatinine ratios increased with age in both genotypes, with significantly higher levels in KO vs. coeval WT mice at 18 (by 31 and 49%, respectively) and 24 (by 55 and 65%, respectively) mo of age (Fig. 1, A and B).

Renal structure. Aged WT mice showed only a modest degree of glomerular and tubulointerstitial damage; conversely, significant renal lesions were detectable in KO mice, particularly at 24 mo of age (Fig. 2). There were PAS-positive deposits within the mesangium, thickening of glomerular basement membrane and Bowman’s capsule (with formation of synechiae and capsular fibrosis), extensive glomerular sclerosis (prevailing at the vascular pole), iailnosis of the afferent arterioles, proteinaceous and hyaline casts in the distal tubuli, and vacuolar degeneration of the cortical tubuli with interstitial fibrosis. All structural parameters increased significantly with
Table 1. Final body weight, serum creatinine, kidney wet weight, GSI, and circulating and renal AGE levels in galectin-3 KO vs. WT mice of 2, 6, 12, 18, and 24 mo of age

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Age, mo</th>
<th>WT</th>
<th>KO</th>
</tr>
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<tbody>
<tr>
<td>2 mo</td>
<td>21.2 ± 1.1 (6)</td>
<td>21.2 ± 1.1 (8)</td>
<td>21.2 ± 1.1 (6)</td>
</tr>
<tr>
<td>6 mo</td>
<td>20.5 ± 1.1 (6)</td>
<td>20.5 ± 1.1 (8)</td>
<td>20.5 ± 1.1 (6)</td>
</tr>
<tr>
<td>12 mo</td>
<td>20.0 ± 1.1 (6)</td>
<td>20.0 ± 1.1 (8)</td>
<td>20.0 ± 1.1 (6)</td>
</tr>
<tr>
<td>18 mo</td>
<td>20.0 ± 1.1 (6)</td>
<td>20.0 ± 1.1 (8)</td>
<td>20.0 ± 1.1 (6)</td>
</tr>
<tr>
<td>24 mo</td>
<td>20.0 ± 1.1 (6)</td>
<td>20.0 ± 1.1 (8)</td>
<td>20.0 ± 1.1 (6)</td>
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Values are means ± SD, no. of mice in parentheses. WT, wild-type; KO, knockout; GSI, glomerular sclerosis index; AGE, advanced glycation end product; RAGE, receptor for advanced glycation end products. Significantly different from the corresponding WT mice at \( P < 0.01 \), \( P < 0.001 \), or \( P < 0.0005 \) for both. Redrawn from Kucharska et al. [6].

AGE-related renal disease in galectin-3 null mice

Age-related changes in kidney structure were similar in the two groups, the amplitude of renal ECM and TGF-β expression was more pronounced in the KO mice (+34–48% at 24 vs. 2 mo of age) than in the WT mice (+31–34%). Transcript levels were significantly higher in KO vs. WT animals starting at 12 mo of age, with maximal increases detected at 24 mo of age (fibrinectin +38%, laminin B1 +46%, collagen IV α1-chain +53%, and TGF-β1 +25%; Fig. 3). Fibrinectin and collagen IV protein expression at the renal/glomerular level also increased with age in both genotypes and was significantly more pronounced in KO than WT mice, particularly at 24 mo of age (Fig. 4).

Circulating and renal tissue AGE levels. Circulating and renal tissue AGE levels increased significantly with age in both genotypes, although increases were more marked in KO vs. WT mice, with differences that were significant at 12 (+26 and 31%, respectively), 18 (+32 and 43%), and, particularly, 24 (+46 and 55%) mo of age (Table 1). Both serum and kidney AGE levels correlated significantly with proteinuria, GSI, and fMMA (Fig. 5) as well as with other parameters of renal function and structure, including albuminuria (\( R^2 = 0.83 \), \( P = 0.000001 \) for both), mG A (\( R^2 = 0.45 \) and 0.64, \( P = 0.0004 \) and 0.0002, respectively), and mM A (\( R^2 = 0.79 \) and 0.85, \( P = 0.000005 \) and 0.0000004, respectively).

Renal AG E receptor gene expression. The kidney cortex gene expression for AGE receptors showed small variations with age in the WT mice, with reduction of OST-48/AGE-R1 (−19%) and increase in 80K-H/AGE-R2 (+21%), galectin-3/AGE-R3 (+28%), RAGE (+22%), and MSR-A type II (+20%). A more marked age-dependent increase in RAGE (+30%), together with similar changes in OST-48/AGE-R1 (−20%), 80K-H/AGE-R2 (−24%), and MSR-A type II (−17%), was detected in the KO mice. Transcripts for 80K-H/AGE-R2 and RAGE were significantly higher at any age (+42–45% and 32–38%, respectively), and those for MSR-A type II were significantly lower starting at 12 mo (−12 to 14%) in KO vs. WT mice (Fig. 6), whereas no significant difference in the mRNA level for OST-48/AGE-R1 was observed between the two genotypes.

Plasma isoprostane 8-epi-PGF2\(_{2\alpha}\) levels. Plasma levels of isoprostane 8-epi-PGF2\(_{2\alpha}\) increased with aging in both genotypes. However, increases were significantly more marked in KO than in WT mice; values were 152.2 ± 14.4 pg/ml at 24 mo vs. 93.4 ± 6.3 at 6 mo (+63%, \( P < 0.001 \)) in KO and 126.5 ± 16.5 vs. 91.2 ± 9.5, respectively (+38%, \( P < 0.01 \)), in the WT animals.

Renal C ML and H NE levels. Renal content of CML and HNE was increased in KO vs. WT mice at 18 and, particularly, 24 mo of age (Fig. 7). In the glomeruli, CML was only barely detectable in KO, but not WT mice, whereas HNE immunoreactivity was more pronounced than that of CML and was
significantly higher in KO vs. WT animals (19.73 ± 3.02 vs. 8.751 ± 2.57% of glomerular area, P < 0.001).

Renal NF-κB activity. The activity of NF-κB p65 within the kidney tissue increased significantly in aged mice from both genotypes. However, transcription factor activity increased more markedly in KO (0.26 ± 0.04 OD at 24 mo vs. 0.17 ± 0.03 at 6 mo, +54%, P < 0.005) than in WT (0.21 ± 0.03 vs. 0.16 ± 0.03, respectively, +31%, P < 0.05) mice.

Fig. 2. Histological appearance of kidney sections from WT and galectin-3 KO mice of 12 (A and B) and 24 (C and D) mo of age [periodic acid-Schiff (PAS), original magnification ×400]. PAS highlights extracellular matrix (ECM) deposits (bright pink stain) in the mesangium (arrows) and thickness of Bowman’s capsule (triangles), which were more pronounced in KO than WT mice. As a result of mesangial expansion, capillary lumen become compromised in KO mice, with capillary occlusion and aneurysm formation (†).
Fig. 3. Renal cortex mRNA levels for fibronectin (FN, A), laminin B1 (LM, B), collagen IV α1-chain (C-IV, C), and transforming growth factor-β1 (TGF-β, D) (expressed as OD ratio to β-actin mRNA level) in galectin-3 KO (filled bars) vs. WT (open bars) mice of 2, 6, 12, 18, and 24 mo of age. Matrix proteins and TGF-β mRNA expression increased significantly with age in both genotypes, but increases were significantly more pronounced in KO than WT mice, starting at 12 mo of age. Significantly different from the corresponding WT mice at *P < 0.001 or †P < 0.01. Comparison among different age groups by 1-way ANOVA was significant at *P < 0.001 for all parameters in both genotypes.

**DISCUSSION**

In these studies, we could extensively characterize the renal functional, structural, biochemical, and molecular changes occurring with age in normal mice and detect similarities and differences with those observed in diabetic (and AGE-injected) animals. Aging mice exhibited progressive increases in total proteinuria, albuminuria, GSI, glomerular area and volume, fractional and absolute mesangial area, circulating and renal tissue AGE and plasma isoprostane 8-epi-PGF_2α levels, kidney CML and HNE content, and renal NF-κB p65 activity. In addition, they showed an age-dependent increase in kidney cortex mRNA level for the ECM proteins fibronectin, laminin, and collagen IV, the prosclerotic cytokine TGF-β1, and the AGE receptors 80K-H/AGE-R2, galectin-3/AGE-R3, RAGE, and MSR-A type II, with reduction of OST-48/AGE-R1 gene expression. Fibronectin and collagen IV protein expression at the renal/glomerular level also increased with age.

The results of our study are in keeping with previous reports in experimental animals, also showing the development of proteinuria, glomerular sclerosis, and tubulointerstitial fibrosis with increasing age (4). In particular, our data are consistent with previous studies in >2-yr-old rats, showing a progressive accumulation of ECM proteins (1) and TGF-β1 (44) and an upregulation of their mRNA levels, indicating an increased matrix synthesis (9). However, the extent of ECM and TGF-β1 mRNA upregulation in our study was lower than that detected in aged rats and closer to that previously observed in diabetic and AGE-injected mice (42, 23). The age-dependent increase in RAGE and AGE-R3/galectin-3 and reduction in OST-48/AGE-R1 transcript level is also consistent with previous reports in aging animals and humans (33, 43, 48), as the progressive increment in circulating and renal tissue AGEs (46, 45), thus supporting the concept that changes in AGE receptor expression are modulated by AGE levels. However, changes in AGE receptors with age were much less pronounced than those of AGE levels, in keeping with the absence of large age-related variations in mRNA expression in rat kidneys (40). Our results are also in agreement with earlier reports showing age-dependent accumulation of CML (45) and HNE (25) and increases in plasma isoprostane 8-epi-PGF_2α levels (39). Finally, the increase in renal NF-κB p65 activity is in keeping with previous data from Fischer 344 rats (27) and also with reports from other tissues of aged animals, including liver (17), brain (30), heart (16), and gastric mucosa (55).

These changes closely resemble those previously detected by our group in mice rendered diabetic with streptozotocin or injected with CML (42, 23), although the extent of increases in serum and kidney AGE levels, renal CML and HNE accumulation, and NF-κB p65 activity was lower in aged mice (also the degree of proteinuria was less). On the other hand, these biochemical abnormalities lasted for a longer period of time than in diabetic and CML-treated mice and paralleled closely the development of changes in renal function and structure, which occurred more slowly since they were fully expressed only at 18–24 mo of age.

We could also confirm the working hypothesis that galectin-3 KO mice are more prone to develop age-dependent renal disease than the WT animals, in keeping with previous reports from our group showing accelerated glomerulosclerosis in diabetic or AGE-injected galectin-3 KO animals vs. their corresponding WT controls (42, 23). This was indicated by the finding that KO mice had more pronounced changes in renal function and structure, associated with more marked increases in circulating and renal tissue AGE levels, plasma isoprostane 8-epi-PGF_2α concentrations, renal content of the glycoxidation and lipoxidation products CML and HNE, and renal NF-κB p65 activity compared with coeval WT mice. KO mice also showed a higher transcript level (and more pronounced age-dependent increases) of 80K-H/AGE-R2 and RAGE, together with lower gene expression (and less marked increment with
age) of MSA-A type II, than coeval WT animals. This altered expression pattern was already detectable at 2 mo of age and was maintained (and further enhanced) throughout the entire life span, thus confirming and extending our previous observations in diabetic and AGE-injected animals (23, 42).

These data demonstrate that age-related changes in renal function and structure are significantly correlated with the biochemical and molecular events associated with activation of the AGE/AGE receptor pathway, i.e., increased AGE levels, AGE receptor expression, ROS generation and oxidative stress, and NF-κB activity (56, 32, 6, 47). This correlation supports the concept of a central pathogenetic role for AGEs and AGE receptor-mediated events in the development of glomerular lesions in aged animals. More importantly, our study provides the novel finding that a more advanced glomerulopathy develops with aging in a model of increased susceptibility to AGE-induced tissue injury, such as the galectin-3 KO mouse (42, 23), in which the events associated with RAGE signaling.
and downregulation by galectin-3 are significantly more pronounced. In fact, galectin-3 seems to interfere with the redox-sensitive pathway triggered by RAGE, either directly or through the modulation of the expression of the other AGE receptors to favor AGE degradation vs. cell activation, thus providing protection toward AGE-dependent tissue injury (22).

Conversely, all of the concurrently assessed functional, structural, biochemical, and molecular parameters were similar in the young KO and WT mice. Taken together, our results provide strong experimental evidence linking AGEs, oxidative stress, and low-grade inflammation in the pathogenesis of age-related renal disease.

Aging and age-related disorders have been associated with chronic low-grade inflammation, although it is unclear whether the increased levels of proinflammatory cytokines and chemokines play a pathogenic role in the process of aging or reflect the young KO and WT mice. Taken together, our results provide strong experimental evidence linking AGEs, oxidative stress, and low-grade inflammation in the pathogenesis of age-related renal disease.

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the presence of associated disorders (8). Aged female mice were found to develop progressive glomerular lesions associated with macrophage infiltration and a gene expression profile of intact glomeruli characterized by upregulation of inflammation-related genes, especially those expressed by activated macrophages, which might result from phenotypic proinflammatory changes in mesangial cells (59). The transcription factor NF-κB appears to play a major role in this scenario, since it participates in the control of cell-cycle, apoptosis, oxidative stress, immunity, inflammation, and repair, all functions that have been found to be profoundly altered during cellular senescence in cell culture systems and the aging process of humans and animals (14). The increased constitutive activity of NF-κB detected in tissues of aged animals (16, 17, 27, 30, 55) as well as in senescent cells (57) strongly supports this concept. In addition, most of the NF-κB target genes were shown to be modulated during senescence or aging by using transcriptome and proteome analysis (14). NF-κB has also been implicated in several renal disorders of an immune and nonimmune nature (15). It is activated by a variety of stimuli,
ranging from cytokines to stress, particularly oxidative stress; there is great evidence that oxidizing conditions in the cytoplasm promote latent NF-κB dimers to be activated (34). AGE binding to RAGE is associated with generation of ROS, at least in part via stimulation of NADPH oxidase (53), and ROS-induced MAPK-dependent activation of NF-κB (32, 6) ultimately leading to tissue injury (47). Both CML (29) and HNE (26) have been shown to be capable of activating NF-κB. Thus this transcription factor seems to be the common final pathway of pathological conditions characterized by inflammation associated with altered cell and ECM turnover (15). These conditions include those in which AGEs play a central pathogenetic role through the AGE/AGE receptor pathway, which is activated by RAGE and downregulated by galectin-3, such as diabetic glomerulopathy and, as indicated by our data, age-related renal disease. It is known that some beneficial effects of angiotensin-converting enzyme inhibitors and statins may, at least in part, be mediated by an inhibition of NF-κB activation (15). A better understanding of the mechanisms involved in NF-κB regulation by AGEs and other pathological stimuli may contribute to the design of novel pharmacological interventions for treatment of several renal (and nonrenal) diseases.

In conclusion, the finding that age-dependent glomerular lesions were more pronounced in galectin-3 KO than WT mice supports the concept that the AGE/AGE receptor pathway (causing oxidative stress and chronic low-grade inflammation) is implicated in the pathogenesis of age-related renal disease and confirms the protective role of galectin-3 as an AGE receptor toward AGE-dependent tissue injury.

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