Alteration of connexin expression is an early signal for chronic kidney disease

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Chronic kidney disease (CKD) can be promoted by a variety of mechanisms including hypertension, diabetes, ischemic, immunological, and toxic injury. These pathologies may affect any of the kidney structures including renal vessels, glomeruli, and the tubulointerstitial compartment. All these events are linked by their common ability to promote development of fibrosis and progressive decline of renal function (7).

Inflammation and excessive scarring are complex processes that involve cell-cell and cell-matrix interactions. These patho-logical conditions have been recently related to disruptions of gap junction-mediated intercellular communication (GJIC) (5, 26, 34). Gap junctions are composed of intercellular channels formed by connexins (Cx) that allow the direct exchange of ions, small metabolites, and other second messenger molecules between adjacent cells (29). Full gap junction channels span two plasma membranes. One gap junction channel results from the docking of two hemichannels or connexons, assembled from six Cx proteins. Hemichannels themselves can also open under both physiological and pathological conditions and this activity may participate in a number of cellular processes (9, 16). Each type of Cx-made channel has unique inherent gating properties or permeabilities to various molecules and ions. Thus Cx composition of gap junction channels appears to determine selectivity among second messengers (15).

Recent studies associated alterations of the expression of the three major vascular Cxs (Cx37, 40, and 43) to the development of chronic vascular inflammatory pathologies such as atherosclerosis (1). An upregulation of Cx43 has also been reported in renal inflammation in humans and rodents (17, 36). Moreover, some studies have reported changes in Cx expression after induction of hypertension in animal models (8). Even if these studies are not always consistent, it seems that in general an increased expression of both Cx40 and 43 is observed in certain arteries after induction of renal hypertension using various procedures in animal models (10, 11, 33). Interestingly, replacement of Cx43 by another Cx isoform was associated with decreased expression and secretion of renin, thus preventing the renin-dependent hypertension that is normally induced in the two kidney-one clip (2K1C) mouse model (12). In addition, recent studies demonstrated the crucial role of Cx40 in blood pressure regulation (19, 20). Although all these studies presume an implication of Cxs in renal disease, the presence of these proteins in fibrotic and inflammatory disease in the kidney has not been documented.

In this study, we investigated whether Cx expression was altered in three different experimental models resulting in pronounced inflammatory response leading to CKD. We first took advantage of a genetic model of hypertensive CKD that overexpresses renin in the liver (RenTg mice). Then, we used the anti-glomerular basement membrane (anti-GBM) mouse model that is a robust tool for studying inflammatory-induced glomerular injury. Finally, we performed unilateral ureteral obstruction (UUO), a model known to induce tubular injury and renal interstitial fibrosis. Our data showed an increase in Cx43 expression at the early stages of the disease in all three models, whereas Cx37 was dramatically reduced in the renal cortex of the injured mice. Alterations in Cx expression were paralleled closely by that of cell adhesion molecules (CAMs), indicating that Cxs are early signals of renal inflammation during the development of CKD.

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METHODS

Animals. Experiments were performed in mice using three different experimental models of nephropathy. The first is a transgenic strain of mouse (RenTg) backcrossed in the genetic background 129SV as already described (2). Briefly, RenTg+/- mice express a renin transgene inserted into a liver-specific locus and driven by a liver-specific promoter/enhancer. The renin coding sequence (Ren2/1d) is a synthetic cDNA consisting of parts of the Ren-2 and Ren-1d genes modified to include glycosylation sites for increased stability, a furin cleavage site to enable prorenin to active renin processing to occur in the liver and allow secretion of active renin into the bloodstream. Thus this transgenic strain expresses renin ectopically at a constant high level in the liver, which leads to elevated mRNA and protein levels of active renin.

In addition, the heterozygous RenTg mice were interbred to generate RenTg+/+ mice. Male RenTg (+/+ and +/-) and wild-type (WT) mice were euthanized at 1.5, 3, and 5 mo. Kidneys were removed, decapsulated, and the cortex was dissected from the medulla. The cortical tissue was then used for RNA extraction and immunohistochemistry.

For the anti-GBM model, SV129 males (3 mo-old) were used to induce passive anti-GBM glomerulonephritis by intravenous administration of a total of 1.5 mg total protein/g body wt, administered over 3 consecutive days as previously described (24). We evaluated renal injury and mRNA expression on day 10. Control mice were perfused with PBS.

Finally, 3-mo-old SV129 male mice underwent UUO. Surgery was performed under general anesthesia after intraperitoneal injection of 100 mg/kg body wt ketamine and 5 mg/kg body wt xylazine. The right ureter was ligated at two separate points through a left-flank incision. Mice were allowed to recover for 6 days before tissue collection. In addition, Cx43+/− mice and their littermates were euthanized after 2 wk of obstructive nephropathy.

All mice were kept in well-controlled animal housing facilities and had free access to tap water and pellet food. All protocols were approved by the French Institutional Committee (INSERM and the University of Pierre et Marie Curie, Paris, France).

Measurement of systolic arterial pressure. Systolic arterial pressure (mmHg) was measured with a tail-cuff sphygmomanometer adapted to the mouse, using an automated system (MC 4000 BP analysis system, Hatteras Instruments, Cary, NC). To avoid variations in blood pressure due to day cycle, all measurements were carried out between 9 and 11 a.m. Animals were acclimated for several days before measurements. Only animals that did not display signals of stress and that showed stable and reproducible values of blood pressure for at least 3 consecutive days were used for blood pressure measurements. Ten measurements from each mouse were taken at 2-min intervals, and then a mean value was determined.

Proteinuria and plasma blood urea nitrogen levels. Proteinuria was assessed using the Pyrogallol red method, utilizing a KONELAB software package. Results with P < 0.05 were considered statistically significant.

RESULTS

Changes in Cx expression in hypertension-induced CDK. To study alterations of Cx expression in the kidney during hyper-

Table 1. List of the primers of different genes and their positions used for real-time-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx37</td>
<td>AGTTTCTGCAGATCGAAGCAGGTA</td>
<td>1049–1071</td>
</tr>
<tr>
<td></td>
<td>AGTTTCTGCAGATCGAAGCAGGTA</td>
<td>1184–1162</td>
</tr>
<tr>
<td>Cx43</td>
<td>GTGCGCGGTCTACTTTGCA</td>
<td>163–140</td>
</tr>
<tr>
<td></td>
<td>GAGATGAGCTGTTGACCTTTGTC</td>
<td>239–219</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>815–833</td>
</tr>
<tr>
<td></td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>1124–1104</td>
</tr>
<tr>
<td>MCP-1</td>
<td>CAGGAGCATCATGTACGTCTTTG</td>
<td>168–148</td>
</tr>
<tr>
<td></td>
<td>CAGGAGCATCATGTACGTCTTTG</td>
<td>228–205</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>177–159</td>
</tr>
<tr>
<td></td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>65–82</td>
</tr>
<tr>
<td></td>
<td>CAGGAGCATCATGTACGTCTTTG</td>
<td>178–1808</td>
</tr>
<tr>
<td>HPRT</td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>1873–1855</td>
</tr>
<tr>
<td></td>
<td>TGGTGGCGAATCGACGAGGAGG</td>
<td>104–122</td>
</tr>
<tr>
<td></td>
<td>CAGGAGCATCATGTACGTCTTTG</td>
<td>193–173</td>
</tr>
<tr>
<td>18S</td>
<td>GAGGCGAAGACATTGGCAAG</td>
<td>992–1011</td>
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<td></td>
<td>GAGGCGAAGACATTGGCAAG</td>
<td>1073–1092</td>
</tr>
<tr>
<td>HSP90</td>
<td>TACTCGGCTTTCCTGCTGCA</td>
<td>76–93</td>
</tr>
<tr>
<td></td>
<td>TACTCGGCTTTCCTGCTGCA</td>
<td>148–130</td>
</tr>
<tr>
<td>RPL 32</td>
<td>GCTGCGACATGTTTTAAGG</td>
<td>29–47</td>
</tr>
<tr>
<td></td>
<td>GCTGCGACATGTTTTAAGG</td>
<td>126–107</td>
</tr>
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Cx, connexin; HSP, heat shock protein.
tension-induced renal disease, we have used RenTg mice. The major advantage of this transgenic model is that renin is produced ectopically in the liver at a constant level, leading to elevated plasma concentration of angiotensin II. We have recently shown that with age, RenTg mice display high blood pressure, associated with progressive renal disease (18). In the present study, we used two different genotypes harboring one (RenTg+/−) or two copies (RenTg+/+) of the renin transgene. Three-month-old RenTg+/− mice displayed elevated systolic blood pressure (SBP; 155 ± 6 compared with 122 ± 11 mmHg for the WT littermates) (Fig. 1A). SBP was significantly higher in RenTg+/+ mice (180 ± 5 mmHg) compared with RenTg+/−. As previously described, both genotypes showed slightly increased proteinuria (18). At this early stage, renal morphology, as revealed by Masson’s trichrome staining, appeared to be normal (data not shown). Quantitative RT-PCR analysis showed a fivefold increase in Cx43 mRNA expression in the 3-mo-old RenTg+/− mice compared with the WT (Fig. 1B). At the same time point, the upregulation of the Cx43 mRNA expression was higher in the RenTg+/+ animals. Immunofluorescence staining for Cx43 was almost negligible in the renal cortex of 3-mo-old WT animals (Fig. 1C). In accordance with the expression of Cx43 mRNA, a slight increase in protein expression was detected in the glomerular capillaries of the RenTg+/− mice (Fig. 1D). The Cx43 increase was more pronounced in the renal cortex of RenTg+/+ animals (Fig. 1E), especially in the peritubular capillaries as there was colocalization of Cx43 immunofluorescence with MECA-32, a marker of the endothelial capillaries (data not shown). In contrast to the Cx43 upregulation, a 5-fold decrease in Cx37 mRNA was observed in the 3-mo-old RenTg+/−, while a 10-fold decrease was measured at 5 mo in both
RenTg+/- and RenTg+/- genotypes (Fig. 1F). Cx40 mRNA expression was upregulated in the renal cortex of the RenTg mice (Fig. 1G) compared with healthy animals. However, no obvious differences were detected by immunofluorescence for Cx40 between WT and RenTg mice, at least at these stages of the disease (data not shown). Immunostaining for Cx37 showed an abundant expression of this protein mainly in the endothelium of renal vessels, glomeruli, and peritubular capillaries of cortical slices of healthy mice (Fig. 1H). Cx37 expression was dramatically decreased in 3-mo-old RenTg+/- mice (Fig. 1I) as well as in the 5-mo-old RenTg+/- animals (data not shown). These data show a dramatic shift in the expression of Cx37 and 43 at the early stages of the hypertensive-induced CKD in mice.

**Alteration of Cx expression in anti-GBM mouse model.** To check whether changes in Cx expression are observed in a model of CDK induced by glomerular injury, we induced the anti-GBM model in SV129 mice. Changes in the expression of Cx37 and 43 were studied in the renal cortex of mice suffering from inflammatory glomerular injury. After 10 days of administration of anti-GBM serum, mice demonstrated higher levels of proteinuria (Fig. 2A), BUN, and serum creatinine than the

![Graphs showing proteinuria, BUN, and creatinine levels](image)

![Images showing protein immunostaining](image)

![Images showing mRNA expression levels](image)

Fig. 2. Alteration of Cx37 and 43 expressions in mice after anti-glomerular basement membrane (GBM) serum administration. Proteinuria (A), blood urea nitrogen (BUN; B), and plasma creatinine (C) were measured in the injured mice after 10 days of serum administration. At the same time point, quantitative RT-PCR analysis in the renal cortex showed increased expression of Cx43 mRNA (E) whereas Cx37 mRNA was not affected (D). Immunofluorescence in renal cortical cryosections showed a marked decrease in Cx37 expression (green) in injured mice (G) compared with the control tissues (F). In contrast, Cx43 was upregulated in the injured tissues (I) compared with the control ones (H). Tissues were counterstained with Evans blue (red). Values are means ± SE. For the PCR experiments, values are expressed as the ratio of the target gene to the internal control gene (HSP90); n = 5 mice from each group. Magnification ×200. GP, glomerular capillaries; PC, peritubular capillaries; VE, vascular endothelium. *P < 0.05, **P < 0.01, ***P < 0.001, anti-GBM vs. control mice.
healthy ones (Fig. 2, B and C, respectively). As expected, immunohistological analysis using Masson’s trichrome staining showed that the development of anti-GBM glomerulonephritis was associated with tubular dilatation and infiltration of inflammatory cells into the renal tissue (data not shown). As illustrated in Fig. 2D, Cx37 mRNA was not affected at this stage of the disease, whereas Cx43 mRNA expression seemed to be significantly upregulated (Fig. 2E). Cx37 immunoreactivity was dramatically decreased in the renal cortex of injured mice compared with noninjected animals (Fig. 2, G and F, respectively), which may reflect alternative posttranscriptional regulation. In contrast, Cx43 immunofluorescence was markedly upregulated in the glomeruli of the injured mice compared with healthy ones (Fig. 2, I and H, respectively). In conclusion, as observed with RenTg mice, there was a marked shift in the expression of Cx37 and 43 in the anti-GBM mouse model from 10 days after the serum injection.

Missregulation of Cx expression after obstructive nephropathy. To study whether alterations in Cx expression could also be observed in a model of tubular injury after obstructive nephropathy, we performed unilateral ureteral ligation in SV129 mice. We first measured mRNAs for Cx37 and Cx43 after 6 days of ureteral ligation. A twofold decrease was observed in Cx37 mRNA expression (Fig. 3A), whereas Cx43 mRNA was significantly upregulated (Fig. 3B). Cx37 protein was mainly expressed in the renal endothelium and also detected at the reticular level of some tubules as already observed in nonoperated animals (data not shown) and as previously reported (13, 32). In accordance with its mRNA expression, immunofluorescence for Cx37 was dramatically reduced in the renal cortex of damaged tissues compared with uninjured controls (Fig. 3, D and C, respectively). In contrast, Cx43 was highly increased in particular in the tubules of the obstructed kidneys (Fig. 3F) compared with the contralateral controls (Fig. 3E). These

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Fig. 3. Alteration of Cx37 and 43 expressions in mice following unilateral ureteral obstruction (UOO). Quantitative RT-PCR analysis in the renal cortex showed a marked decrease in Cx37 mRNA expression (A) whereas Cx43 mRNA (B) was increased after 6 days of obstructive nephropathy. Confocal microscopic images (green) of Cx37 (C and D) and Cx43 (E and F) were obtained from cryosections of control (C and E) and injured mice (D and F). Tissues were counterstained with Evans blue (red). Values are means ± SE and expressed as the ratio of the target gene to the internal control gene (HPRT); n = 7 mice from each group. Magnification ×400. TC, tubular cell. **P < 0.01, UOO vs. control tissues.
results showed misregulation of both Cx37 and 43 expressions in mice from 6 days of obstructive nephropathy.

**CAM expression in hypertension-induced CDK.** Alterations of Cx expression have been related to inflammation and endothelial dysfunction in chronic vascular diseases (1). Consequently, we measured CAM expression known to play a major role in inflammatory cell recruitment and studied macrophage infiltration. Quantitative RT-PCR showed an almost threefold increase in VCAM-1 mRNA in the renal cortex of RenTg+/− mice. The VCAM-1 upregulation was higher in RenTg+/+/ (Fig. 4A). ICAM-1 mRNA was also increased, by almost twofold, in RenTg+/− since 3 mo, and its upregulation was more pronounced in the RenTg+/+/ mice (Fig. 4B). CD68 immunostaining was negligible in the renal cortex of WT and RenTg+/− 3-mo-old mice (Fig. 4, C and D, respectively). In contrast, a moderate macrophage infiltration was detected in cortical slices of RenTg+/+ 3-mo-old mice (Fig. 4E). In conclusion, the Cx43 mRNA expression pattern was paralleled closely by that of VCAM-1 and ICAM-1 and preceded inflammatory cell infiltration.

**CAM expression in anti-GBM and obstructive nephropathy.** CAM expression was also measured by RT-PCR using the renal cortex of mice after 10 days of injection of anti-GBM antibodies and 6 days of UUO. VCAM-1 and ICAM-1 mRNAs were markedly increased in the renal cortex of the anti-GBM mice (Fig. 5, A and B, respectively). Moreover, the mRNA of the chemoattractant factor MCP-1 was also upregulated by 100-fold (Fig. 5C).

In the UUO model, a significant increase was observed, by at least sevenfold, of the mRNA of adhesion markers such as VCAM-1, ICAM-1, and P-selectin (Fig. 5, D, E, and F, respectively) compared with contralateral nonobstructed kidneys. Thus our data show that changes in the equilibrium between Cx37 and 43 are accompanied by induction of adhesion molecules preceding renal inflammation in the three experimental models independently of the originating cause.

**Decreased Cx43 expression downregulates CAM expression following obstructive nephropathy.** To assess whether Cx43 may participate in renal inflammation, we studied VCAM expression in Cx43 heterozygous mice after 15 days of ob-

![Figure 4](image-url)

**Fig. 4.** Study of inflammatory indexes in RenTg mice. Quantitative RT-PCR for VCAM-1 (A) and ICAM-1 (B) are presented as means ± SE and expressed as the ratio of the target gene to the internal control gene (18S rRNA); n = 5, 8, and 8 for WT, RenTg+/−, and RenTg+/+ mice, respectively (**p < 0.01, ***p < 0.001 vs. WT). CD68 immunostaining was performed using renal cortical slices from 3-mo-old WT (C), RenTg+/− (D), and RenTg+/+ (E) mice. Macrophage infiltration was only detected in RenTg+/+ animals. Magnification ×200.
structive nephropathy. Figure 6A shows that Cx43 mRNA was still significantly increased after 15 days of UUO compared with the contralateral nonobstructive kidneys. As expected, Cx43 mRNA expression was reduced in Cx43−/− control mice. Following UUO, Cx43 upregulation was less compared with WT. In addition, VCAM-1 mRNA was highly increased in WT animals after 15 days of UUO whereas this upregulation was significantly restricted in Cx43 heterozygous animals (Fig. 6B). These data suggest that decreased Cx43 expression blunts the upregulation of markers of the recruitment of inflammatory cells. Consequently, the upregulation of this Cx may participate in the inflammatory response during tubulointerstitial renal injury.

DISCUSSION

Alteration of Cx expression has been recently reported in some acute and chronic inflammatory models in mice (28). The aim of our study was to investigate whether Cx expression is altered during the development of CKD. For this purpose, we used three different experimental models that are linked by their common ability to promote inflammatory reactions and injury at distinct renal compartments. Among approximately 10 Cx subtypes known to be expressed in the kidney, we decided to investigate the expression of the 3 major vascular Cxs (Cx37, Cx40, and Cx43), since previous studies indicate a link between the expression of these Cxs and the development of vascular inflammation (3, 4, 22, 35).

We first used the hypertension-induced renal disease RenTg model that mimics more closely the kinetics and characteristics of human nephroangiosclerosis (18). These mice are hypertensive and display moderate proteinuria from 3 mo of age (data not shown). However, these pathological features are accentuated with age and accompanied by structural alterations of renal morphology. These alterations took place in 5-mo-old RenTg+/− mice simultaneously with macrophage infiltration as observed in the renal cortex of these animals. Moreover, Sirius red staining showed the beginning of renal interstitial fibrosis. Pronounced tubular dilatation was detected at 7 mo (data not shown). The disease was more severe in the RenTg−/− animals as the beginning of macrophage infiltration was observed as early as 3 mo (Fig. 4), and massive inflammatory infiltration and tubular dilatation were detected in the 5-mo-old animals (data not shown). In these animals, Cx43 expression was increased since 1.5 mo and preceded the increase in CAM mRNA. The Cx43 upregulation was more pronounced, especially in the peritubular capillaries in RenTg−/− compared with RenTg+/− mice, in which renovascular disease seemed moderate. These data suggest that increased expression of Cx43 is an early event associated with subsequent increased expression of major CAMs, indicating that an inflammatory predisposition is on the way at the early stages of hypertension-induced renal disease.

The endothelium plays a crucial role in the orchestration of inflammation. Under pathological conditions, inflammatory cells migrate from vessels into tissues by a multistep process involving the sequential activation of CAMs and their ligands, both on inflammatory and endothelial cells. Thus, in the response to injury, the endothelium becomes dysfunctional, leading to increased expression of various CAMs that are necessary for the recruitment of inflammatory cells. The crucial role of endothelial dysfunction has been demonstrated in chronic vascular inflammatory pathologies such as atherosclerosis and has been interpreted as the starting point of this disease (14). However, the implication of endothelial dysfunction in renal disease seems underestimated.

There is increasing evidence of the importance of Cxs in modulating the severity of inflammatory disease. An increased expression of the Cx43 has also been reported during the
connexin 43 may serve as a conducting pathway by amplifying calcium inflammatory response (31). In addition, it has been suggested that greatly reduced, pointing to a critical role of this connexin in the TNF-α/H9251

Cameral form of Cx43 in leukocyte adhesion has been recently reported using a leading to moderate vascular smooth muscle cell activation, as a result of decreased macrophage infiltration in the injured site, /H11002

mates LDLR /H11002

angioplasty of the carotid artery, compared with control littermates /H11002

atherosclerosis in mice (22). In addition, atherosclerotic plaque development and blunted changes in the course of atherosclerotic-related diseases in mice (22). In addition, atherosclerosis-susceptible mice expressing reduced levels of Cx43 (LDLR−/−Cx43+/−) displayed restricted intimal thickening, following angioplasty of the carotid artery, compared with control littermates LDLR−/−Cx43+/+ mice (3). The reduction of Cx43 resulted in decreased macrophage infiltration in the injured site, leading to moderate vascular smooth muscle cell activation, associated with accelerated vascular repair. An important role of Cx43 in leukocyte adhesion has been recently reported using a Cx43 endothelium-specific deletion in the mouse. In this model, TNF-α-induced leukocyte adhesion and transmigration were greatly reduced, pointing to a critical role of this Cx in the inflammatory response (31). In addition, it has been suggested that Cx43 may serve as a conducting pathway by amplifying Ca2+ signaling between endothelial cells to spread inflammatory signals within the lung capillary network (25). Finally, Cx43+/− mice displayed reduced neutrophil recruitment to the lung after intratracheal instillation of LPS (27). Consequently, Cx43 may participate in endothelial dysfunction as it seems to be implicated in the adhesion of leukocytes.

Then, we used the anti-GBM model to study Cx expression in inflammatory renal injury. This model exhibits linear deposition of IgG along the GBM, leading to severe histological and functional injury including proteinuria. This model implicates an exacerbated secretion of proinflammatory cytokines and chemokines responsible for the early upregulation of adhesion molecules such as ICAM-1 and VCAM-1. In this model, Cx37 expression was dramatically decreased in the renal cortex. In contrast, Cx43 immunoreactivity was strikingly increased along the glomerular capillary wall and to a lesser extent in the peritubular capillaries at the early stages of nephritic disease. Pronounced Cx43 immunostaining was also observed in the dilated tubules after 15 days of disease (data not shown).

Finally, we used the UUO model which initiates a rapid sequence of events in the obstructed kidney, leading, within 24 h, to reduced renal blood flow and renal filtration rate (30). This is followed by interstitial macrophage infiltration, tubular cell death, and hydrenephrosis. In this model, changes in Cx43 and 37 expressions followed a similar pattern as in the previous experimental models. Alteration of Cx43 expression was similar to those of CAMs. Interestingly, Cx43 expression was upregulated in atrophic tubules and colocalized with CAMs in biopsies from patients with inflammatory renal disease. This study suggests that Cx43 may be an important mediator of pathological conditions in the kidney, as its upregulation on the surface of tubular and interstitial cells will facilitate their interaction with infiltrating leukocytes (17). To assess whether modulation of Cx43 expression could alter the expression of CAM during the development of CDK, Cx43 heterozygous mice underwent UUO and were euthanized after 2 wk of tubulointerstitial injury, a time point known to present severe damage in the renal tissue. Interestingly, Cx43+/− mice blunted activation of VCAM-1, indicating that Cx43 participates in inflammation during the development of kidney disease.

mRNA expression of Cx40 was also increased in the three experimental mouse models, as has already been reported in various renovascular models (1). Nevertheless, immunoreactivity of Cx40 was similar between healthy and injured tissues. Additional experiments using Cx40-deficient mice will provide more information about the role of this Cx in the inflammatory process during the development of CDK.

In sharp contrast to Cx43, Cx37 expression was dramatically decreased at later time points in RenTg mice, and this decrease occurred at the same time as the interstitial macrophage infiltration. A similar shift between Cx37 and 43 expression has been reported during atherosclerotic plaque formation (6, 21). Interestingly, deletion of Cx37 accelerated atherosclerosis in hypercholesterolemic mice, indicating that in contrast to Cx43, this Cx plays a protective role against the disease by regulating monocyte adhesion (35).

CAMs mediate the interaction between inflammatory cells and intrinsic cells within tissues (23). In renal disease, they are thought to dictate the interaction between infiltrating and endothelial cells, thus enabling the local delivery of cytokines and facilitating intercellular signaling. The associated expression of Cx could allow second messengers and other signals to be directly delivered. In addition, adhesion is essential for stable cell-to-cell interactions, which include the formation of gap junctions, and there is increasing evidence that the two processes are mutually dependent. Indeed, interactions be-
between connexons are a form of intercellular adhesion. Although further work is required to clarify the relationship between adhesion molecules and Cx43 in the kidney and to define the role of alternative Cxs, our study shows that alteration of Cx expression can be a new important biomarker to detect early changes leading to CKD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


