Loss of $\alpha_3/\alpha_4(IV)$ Collagen from the Glomerular Basement Membrane Induces a Strain-Dependent Isoform Switch to $\alpha_5\alpha_6(IV)$ Collagen Associated with Longer Renal Survival in Col4a3$^{-/-}$ Alport Mice

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Mutations in COL4A3/4/5 genes that affect the normal assembly of the $\alpha_3/\alpha_5/\alpha_6(IV)$ collagen network in the glomerular basement membrane (GBM) cause Alport syndrome. Patients progress to renal failure at variable rates that are determined by the underlying mutation and putative modifier genes. Col4a3$^{-/-}$ mice, a model for autosomal recessive Alport syndrome, progress to renal failure significantly slower on the C57BL/6 than on the 129X1/Sv background. Reported here is a novel strain-specific alternative collagen IV isoform switch that is associated with the differential renal survival in Col4a3$^{-/-}$ Alport mice. The downregulation or the absence of $\alpha_3(IV)$ collagen chains in the GBM of LmxAb$^{-/-}$ and Col4a3$^{-/-}$ mice was found to induce ectopic deposition of $\alpha_5(IV)$ collagen. The GBM deposition of $\alpha_5(IV)$ collagen was abundant in C57BL/6 Col4a3$^{-/-}$ but almost undetectable in 129X1/Sv Col4a3$^{-/-}$ mice. This strain difference was due to overall low expression of $\alpha_6(IV)$ chain and $\alpha_5(IV)$ protomers in the tissues of 129X1/Sv mice, a natural Col4a6$^{-/-}$ knockdown. In (129 × B6)F1 Col4a3$^{-/-}$ mice, the amount of $\alpha_5(IV)$ collagen in the GBM was inherited in a mother-to-son manner, suggesting that it is controlled by one or more X-linked loci, possibly Col4a6 itself. Importantly, high levels of ectopic $\alpha_5(IV)$ collagen in the GBM were associated with approximately 46% longer renal survival. These findings suggest that $\alpha_5(IV)$ collagen, the biologic role of which has been hitherto unknown, may partially substitute for $\alpha_3/\alpha_4/\alpha_5(IV)$ collagen. Therapeutically induced GBM deposition of $\alpha_5(IV)$ collagen may provide a novel strategy for delaying renal failure in patients with autosomal recessive Alport syndrome.


Received February 22, 2006. Accepted April 15, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1707-1962
supporting normal glomerulogenesis and providing adequate functionality early in life, the Alport GBM gradually develops characteristic ultrastructural abnormalities, and patients progress slowly but inexorably to renal failure that requires renal replacement (9). The X-linked and autosomal recessive forms of disease are clinically similar but distinguished by the inheritance mode and biochemically. In X-linked Alport syndrome, COL4A5 mutations prevent the normal assembly of both α3α4α5(IV) and (α5)2α6(IV) protomers. In autosomal recessive Alport syndrome, mutations in the COL4A5 gene prevent the expression of collagen IV chains and their specific assembly into networks and how pathogenic mutations interfere with these processes remain poorly understood. Instrumental for addressing these questions are animal models that closely reproduce the genetic, biochemical, and clinical features of human disease. The most widely used model, Col4a3−/− mice, develop a renal phenotype similar to autosomal recessive Alport syndrome (14,15). Also useful are animal models that exhibit dysregulated expression of collagen IV chains, such as Lmx1b−/− mice (16), a model of nail-patella syndrome. The Col4a3/Col4a4 genes are strongly downregulated in Lmx1b−/− mice, preventing the expression of α3 and α4(IV) collagen chains, yet the α5(IV) chain persists in the mutant GBM (16). This paradoxical uncoupling between the α5(IV) chain and α3/α4(IV) chains challenges the current paradigm of the specific assembly and tissue-restricted expression of collagen IV chains. To address this incongruency, we studied the distribution of collagen IV chains in the kidneys of several lines of knockout mice with GBM defects. The findings revealed a new alternative collagen IV isoform switch, whereby α5/α6(IV) collagen is deposited ectopically in the GBM of mutant mice that lack the α3/α4(IV) collagen chains. We report the biochemical basis, the characteristic ultrastructural abnormalities, and patients

**Materials and Methods**

Mouse mAb 8D1 reacted specifically with human and rodent α3NC1 monomers and α3NC1-containing hexamers. Rat mAb RH42 (anti-α4NC1 [17]), b14 (anti-α5NC1 [18]), and B66 (anti-α6NC1 [4]) were used for immunofluorescence staining, and affinity-purified rabbit polyclonal antibodies were used for detection of α1/2(IV) collagen. Rat mAb M54 and M69 (19) were used for specific detection of murine α5 and α6 NC1 domains in Western blots; other chain-specific mAb were as described (17). mAb 8D1 and b14 coupled to Affigel-10 were used for separation of NC1 hexamers that contained α3 and α5 subunits, respectively.

**Animals**

Congenic mice with a targeted mutation of Col4a3 gene were generated by backcrossing onto the C57BL/6J (B6) and 129X1/SvJ (129) backgrounds (20). A distinct line of 129X1-Col4a3<sup>Lam2Δ5</sup>−/− mutant mice was purchased from Jackson Laboratories. Col4a3 mutant mice were maintained under standard conditions in pathogen-free animal care facilities. Lamb2-null and Lmx1b-null mice on a mixed (129 × B6) genetic background were described previously (21,22). All mouse experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees at each participating institution.

**Indirect Immunofluorescence Staining**

Snap-frozen mouse tissues that were embedded in OCT were cut into 5-μm cryosections, fixed in acetone for 10 min at −20°C, blocked with 1% BSA, and incubated with primary antibodies for 2 h at 37°C, followed by fluorophore-conjugated secondary antibody for 1 h at room temperature in the dark. Sections were mounted in gelmount (Biomedia Corp., Foster City, CA) and examined under a Nikon Eclipse E800 fluorescence microscope. Photomicrographs were recorded with a charge-coupled device digital camera, using the same exposure settings for each primary antibody.

**Western Blot and Immunoaffinity Fractionation**

Basement membranes that were isolated from mouse kidneys, lungs, and bladders by detergent extraction were digested overnight with bacterial collagenase (Calbiochem, San Diego, CA). Solubilized NC1 hexamers were separated by SDS-PAGE in 6 to 20% gradient gels under nonreducing conditions and then transferred to Immobilon P membranes. For detection of NC1 domains, the membranes were blocked with 5% casein, incubated overnight with specific mAb, then stained with alkaline phosphatase–conjugated secondary antibody followed by fluorophore-conjugated secondary antibody for 1 h at room temperature in the dark. Sections were mounted in gelmount (Biomedia Corp., Foster City, CA) and examined under a Nikon Eclipse E800 fluorescence microscope. Photomicrographs were recorded with a charge-coupled device digital camera, using the same exposure settings for each primary antibody.

**Statistical Analyses**

Statistical analyses were performed with GraphPad Prism version 3.0 (GraphPad, San Diego, CA). Differences among multiple groups were determined by one-way ANOVA followed by post hoc t test with Bonferroni correction for pairwise comparisons.

**Results**

**α5α6(IV) Collagen Chains Are Deposited Ectopically in the GBM of Lmx1b−/− Mice but not Lamb2−/− Mice**

The unexplained persistence of α5(IV) collagen in the GBM of Lmx1b<sup>−/−</sup> mice despite the loss of α3/α4(IV) chains poses a conundrum. Possible explanations are the synthesis of abnormal collagen IV protomers that contain new chain combinations, such as the hypothetical α1/α5(IV) or α1/α2/α5(IV) (23),
or de novo expression of the α6(IV) chain allowing assembly of “canonical” α5α6(IV) protomers at an unusual site. To distinguish between these alternatives, we examined the distribution of collagen IV chains in the kidneys of newborn Lmx1b mutant mice by indirect immunofluorescence staining with chain-specific mAb (Figure 2). In Lmx1b−/− kidneys, the staining for α3 and α4(IV) chains was faint and segmental, but α5(IV) chain persisted in the GBM, in agreement with previous reports (16). Importantly, α6(IV) collagen co-localized with α5(IV) collagen not only in Bowman’s capsule basement membrane (as expected) but also in the GBM of Lmx1b−/− mice. Littermate Lmx1b+/- mice, which have a normal renal phenotype, exhibited a normal distribution of collagen IV chains in the kidneys, defined by the presence of α3(IV) and α4(IV) collagen restricted to the Bowman’s capsule basement membrane, and α5(IV) collagen co-localizing with α3(IV) and α6(IV) chains at these sites (19,24). Therefore, the persistence of α5(IV) chain in the Lmx1b−/− GBM is due to ectopic expression of α6(IV) chain.

To determine whether the ectopic deposition of α6(IV) collagen in the murine GBM is triggered nonspecifically by defective glomerular permselectivity and/or nephrotic-range proteinuria, we evaluated the distribution of collagen IV chains in the kidneys of 4-wk-old nephrotic mice (at 4, 8, 12, 17, and 23 wk of age), mAb to α5(IV) and α6(IV) chains stained strongly both the Bowman’s capsule basement membrane and proteinuria and death by 4 wk of age (21). A normal distribution of collagen IV chains was found in the kidneys of both nephrotic Lamb2−/− and control Lamb2+/+ mice (Figure 3). This indicates that the ectopic expression of α6(IV) collagen in the Lmx1b−/− GBM is not a consequence of defective glomerular permselectivity but may be caused by the loss of Lmx1b transcription factor or secondary effects of this loss on downstream genes, such as Col4a3/Col4a4.

**Ectopic Expression of α5/α6(IV) Collagen Chains in the GBM of Col4a3−/− Mice Is Influenced by Genetic Background**

To determine whether the ectopic expression of α6(IV) collagen in the mouse GBM is caused specifically by the absence of the normal α3α4α5(IV) collagen network, we studied Col4a3−/− mice, the most widely used animal model of autosomal recessive Alport syndrome. In the original studies of Col4a3−/− mice (14,15) or Col4Δ3-4 mice with an insertional inactivation of the common Col4a3/Col4a4 promoter (25), the α3, α4, and α5(IV) collagen chains were not detected in the GBM by staining with polyclonal antibodies, and the expression of α6(IV) chain was not investigated. Therefore, the distribution of α5 and α6(IV) collagen chains in the kidneys of congenic B6 and 129 Col4a3−/− mice was reevaluated here using more sensitive and specific mAb (Figure 4).

We report for the first time that Col4a3−/− mice deposit both α5 and α6(IV) chains in their GBM, despite the loss of α3 and α4(IV) chains. In all kidneys from B6 Col4a3−/− mice (at 4, 8, 12, 17, and 23 wk of age), mAb to α5(IV) and α6(IV) chains stained strongly both the Bowman’s capsule basement membrane and

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**Figure 2.** Indirect immunofluorescence analysis of collagen IV chains expressed in the kidneys of Lmx1b mutant mice. Frozen kidney sections from littermate Lmx1b−/− (A through D) and Lmx1b+/- (E through H) mice were stained with antibodies specific for the α1/α2 (A and E), α4 (B and F), α5 (C and G), and α6 (D and H) collagen IV chains. Note the positive staining for α5(IV) and α6(IV) collagen chains in the glomerular basement membrane (GBM) of Lmx1b−/− mice (arrows in G and H). Magnification, ×600.

**Figure 3.** Indirect immunofluorescence analysis of collagen IV chains expressed in the kidneys of Lamb2 mutant mice. Frozen kidney sections from littermate Lamb2+/+ (A through C) and Lamb2−/− (D through F) mice were stained with antibodies specific for the α4 (A and D), α5 (B and E), and α6 (C and F) collagen IV chains. All collagen IV chains were expressed in a normal pattern in both control and mutant kidneys. Magnification, ×600.
In the kidneys of the control Col4a3 mutant mice, downregulation or the absence of Col4a3 resulted in a lack or absence of the Col4a3 basement membrane and was expressed at higher levels in B6 kidneys. Note the very strong staining for Col4a3 in the kidneys of mice with Col4a3+/−. The amount of Col4a3 expression in the kidneys of 129 mutant mice with distinct mutations varied with strain and age. The normal distribution of collagen IV chains in the GBM and Bowman’s capsule basement membrane of B6 Col4a3−/− mice (G and H), contrasting with much fainter staining of these sites in 129 Col4a3−/− mice (O and P). The pattern of staining for the α5 and α6(IV) collagen chains in the 129 Col4a3−/− kidney was more clearly revealed using longer exposure times (Q and R). Magnification, ×600.

Figure 4. Indirect immunofluorescence analysis of collagen IV chains expressed in the kidneys of Col4a3 mutant mice. Frozen kidney sections from B6 Col4a3+/− (A through D), B6 Col4a3−/− (E through H), 129 Col4a3+/− (I through L), and 129 Col4a3−/− (M through R) mice were stained with antibodies specific for the α1/α2 (A, E, I, and M), α4 (B, F, J, and N), α5 (C, G, K, O, and Q), and α6 (D, H, L, P, and R) collagen IV chains. Note the very strong staining for α5 and α6(IV) collagen chains in the GBM and Bowman’s capsule basement membrane of B6 Col4a3−/− mice (G and H), contrasting with much fainter staining of these sites in 129 Col4a3−/− mice (O and P). The pattern of staining for the α5 and α6(IV) collagen chains in the 129 Col4a3−/− kidney was more clearly revealed using longer exposure times (Q and R). Magnification, ×600.

The organization of collagen IV chains in the kidneys of B6 Col4a3+/− and Col4a3−/− mice was investigated by Western blot (Figure 5). The α1 and α2 NC1 domains (derived from α1α2[IV] protomers) were present at comparable levels in all kidneys and served as loading controls. The α3, α4, and α5 NC1 domains (derived from α3α4α5[IV] protomers) were abundant in Col4a3−/− kidneys, whereas the staining for the α6 NC1 domain was comparatively faint (and weaker in 129 than in B6 kidneys). In the Col4a3−/− mouse kidneys, the α3 and α4 NC1 domains were absent. On both backgrounds, mutant kidneys expressed less α5(IV) but more α6(IV) collagen than the respective control Col4a3+/− kidneys. Comparing the two mutant strains, the staining for α5NC1 and α6NC1 domains was more intense in B6 than in 129 Col4a3−/− kidneys. Therefore, the results of the Western blot analysis fully corroborate the findings of the immunofluorescence study.

Organization of Collagen IV Chains in the Kidneys of B6 Col4a3+/− and Col4a3−/− Mice

To determine whether the α5 and α6(IV) chains co-assemble into triple-helical protomers in the kidneys of Col4a3−/− mice, we analyzed the NC1 hexamers that were solubilized from the kidney basement membranes of control and affected B6 mice, using immunoaffinity separation on chain-specific mAb (Figure 6). Upon analysis of NC1 hexamers from B6 Col4a3+/− mouse kidneys, the α3, α4, and α5NC1 domains were bound quantitatively to immobilized mAb against α3NC1 and α5NC1 domains.

Figure 5. Western blot analysis of collagen IV chains expressed in the kidneys of Col4a3 mutant mice. Collagenase-solubilized renal basement membranes from B6 Col4a3+/−, B6 Col4a3−/−, 129 Col4a3+/−, and 129 Col4a3−/− mice were separated by SDS-PAGE on a 4 to 20% gradient gel under nonreducing conditions and blotted with mAb that specifically recognize the α1 to α6(IV) NC1 domains. D, NC1 dimers (molecular mass of approximately 44 to 50 kD); M, NC1 monomers (molecular mass of approximately 24 to 28 kD).
domains, along with small amounts of \( \alpha_1 \) and \( \alpha_2 \)NC1 domains, whereas the unbound fraction contained only \( \alpha_1 \) and \( \alpha_2 \)NC1 domains. This indicates the presence of \( \alpha_3 \alpha_4 \alpha_5 \alpha_6 \) (IV) protomers, assembled with each other or with \( \alpha_1 \alpha_2 \) (IV) protomers, as previously shown in wild-type mice (17). In contrast, upon analysis of NC1 hexamers from \( \text{Col4a3}^{-/-} \) mouse kidneys, no material was bound by the \( \alpha_3 \)NC1 mAb, whereas the \( \alpha_5 \)NC1 mAb co-precipitated quantitatively the \( \alpha_5 \) and \( \alpha_6 \) (IV) NC1 domains, accompanied by some \( \alpha_1 \) and \( \alpha_2 \) NC1 domains. These findings reveal that in the B6 \( \text{Col4a3}^{-/-} \) kidneys, the \( \alpha_5 \) and \( \alpha_6 \) (IV) chains co-assemble into \( \alpha_5 \alpha_6 \) (IV) protomers, which may interact with \( \alpha_1 \alpha_2 \) (IV) protomers to form an \( \alpha_1 \alpha_2 / \alpha_5 \alpha_6 \) (IV) network, as in bovine (4) and human basement membranes (5).

**129 Mice Have Lower Expression of \( \alpha_6 \) (IV) Collagen than B6 Mice**

To determine whether the low amount of \( \alpha_5 \alpha_6 \) (IV) collagen in the GBM of 129 \( \text{Col4a3}^{-/-} \) mice simply reflects an inadequate compensatory isoform switch in the kidneys or is due to an overall low expression of \( \alpha_6 \) (IV) collagen in 129 mice, we compared B6 and 129 mouse tissues that were rich in smooth muscle basement membranes, where the \( \alpha_5 \alpha_6 \) (IV) network is normally expressed (Figure 7). In \( \text{Col4a3}^{-/-} \) lungs, the \( \alpha_6 \) (IV) expression was lower in 129 than in B6 mice, whereas the \( \alpha_1 \) through \( \alpha_5 \) (IV) chains stained with equal intensity. As expected, in \( \text{Col4a3}^{-/-} \) lungs, only the \( \alpha_1, \alpha_2, \alpha_5 \), and \( \alpha_6 \) NC1 domains were detected, consistent with the presence of \( \alpha_1 \alpha_2 \) (IV) and \( \alpha_5 \alpha_6 \) (IV) protomers and the absence of \( \alpha_3 \alpha_4 \alpha_5 \) (IV) protomers. Notably, both \( \alpha_5 \) and \( \alpha_6 \) NC1 domains were significantly lower in 129 than B6 \( \text{Col4a3}^{-/-} \) lungs, indicating that low levels of \( \alpha_6 \) (IV) chain reduce the assembly of \( \alpha_5 \alpha_6 \) (IV) protomers (Figure 7A).

Similar results were found by Western blot analysis and immunofluorescence staining of bladder basement membranes (Figure 7B). These findings reveal that, regardless of the expression of \( \alpha_3 / \alpha_4 \) (IV) chains, 129 mice have overall lower expression of \( \alpha_6 \) (IV) collagen and \( \alpha_5 \alpha_6 \) (IV) protomers than B6 mice.

**X-Linked Loci Influence Ectopic Deposition of \( \alpha_5 \alpha_6 \) (IV) Collagen in the GBM and Renal Survival in F1 \( \text{Col4a3}^{-/-} \) Mice**

We hypothesized that the reduced expression of \( \alpha_6 \) (IV) collagen in 129 mice may be due to cis-acting elements in the \( \text{Col4a6} \) locus, located on the mouse chromosome X. If so, then the amount of ectopic \( \alpha_5 / \alpha_6 \) (IV) collagen that was deposited in the GBM of (B6 × 129)F1 hybrid \( \text{Col4a3}^{-/-} \) mice would be inherited in a mother-to-son manner, characteristic of X-linked transmission. Consistent with this prediction, the intensity of staining for \( \alpha_5 / \alpha_6 \) (IV) collagen chains in the GBM of F1 mutant mice was strong in \( \text{Col4a3}^{-/-} \) male mice that were born to B6 dams but barely detectable in \( \text{Col4a3}^{-/-} \) male mice that were born to 129 dams (Figure 8, top). In F1 \( \text{Col4a3}^{-/-} \) female mice,
the GBM staining for α5/α6(IV) collagen was in general strong, B6-like; however, many glomeruli exhibited areas of fainter 129-like staining for α5α6(IV) collagen—a mosaic pattern that is consistent with the inactivation of chromosome X. These findings strongly suggest that the expression of the α6(IV) chain—and, therefore, the deposition of (α5)α6(IV) protomers—is controlled by one or more X-linked loci, among which Col4a6 itself is a likely candidate.

Differences in the GBM composition among F1 Col4a3−/− mice raised the possibility that other associated changes in their renal phenotype may occur. An analysis of the renal survival data from a previous study of F1 Col4a3−/− mice (20) showed (Figure 8, bottom) that F1 Col4a3−/− male mice that were born to 129 dams reached ESRD at 80 ± 7.8 d (mean ± SD), which was significantly earlier than F1 Col4a3−/− male mice that were born to B6 dams (114 ± 14.1 d; P < 0.001) or F1 Col4a3−/− female mice (119 ± 7.7 d; P < 0.001). Therefore, high levels of α5/α6(IV) collagen in the GBM of Col4a3−/− mice were associated with approximately 46% longer renal survival, suggesting that the alternative isoform switch may partially compensate for the loss of α3α4α5(IV) collagen.

Discussion

This study demonstrates a novel alternative collagen IV isoform switch in the murine GBM. Despite partial loss of α3 and α4(IV) chains in Lmx1b−/− mice or the complete absence of these chains in Col4a3−/− mice, α5(IV) collagen persists in the affected GBM but is accompanied by de novo deposition of α6(IV) collagen. Ectopic α6(IV) collagen was not detected in other nephrotic kidneys, such as those from Lamb2−/− mice, suggesting that the alternative isoform switch is triggered by the failure of the normal developmental switch to α3α4α5(IV) collagen. We present the first biochemical evidence that murine α5 and α6(IV) chains co-assemble into (α5)α6 protomers, which are part of an α1α2/α5α6(IV) network, as previously found in bovine (4) and human (5) tissues. Studies of human and bovine basement membranes have shown that the six collagen IV chains assemble specifically into exactly three kinds of protomers; our study validates this paradigm for mouse collagen IV chains.

Although ectopic α5α6(IV) collagen was found in the GBM of Col4a3−/− Alport mice on both B6 and 129 genetic backgrounds, there were important quantitative differences between strains. Whereas α5α6(IV) collagen was abundant in the B6 Col4a3−/− GBM, α1α2(IV) collagen remained the predominant GBM network in 129 Col4a3−/− mice. This strain dependence and the lower sensitivity of previously used polyclonal antibodies may explain why the novel isoform switch has been overlooked (commercially available Col4a3−/− mice are now on the 129 background, and in older studies they were on a mixed 129 × B6 background). The difference between strains was unrelated to the presence or the absence of α3/α4(IV) chains but was attributable to the overall low expression of α6(IV) collagen and α5α6(IV) protomers in the tissues of 129 mice, which could be regarded as a natural Col4a6 knockdown. The mechanisms that are responsible for different levels of α6(IV) expression among mouse strains are being investigated.

The alternative switch to α5α6(IV) collagen in the GBM of mice that are deficient in α3/α4(IV) chains, together with quantitative differences between B6 and 129 strains, provide a new experimental model that is uniquely suited for comparing the phenotypic effects of the three canonic collagen IV networks at the same site, namely the GBM. Collagen IV forms the structural backbone of GBM, which counteracts the high transmural hydrostatic pressure gradient that is required for ultrafiltration (26). This role is normally fulfilled by the α3α4α5(IV) collagen network, which is reinforced by numerous intermolecular cross-links (27). Neither the persistence of embryonic α1α2(IV) collagen nor the switch to α5α6(IV) collagen can fully compensate for the loss of α3α4α5(IV) collagen in the Alport GBM, because all strains of Col4a3−/− mice eventually develop renal failure. However, α5α6(IV) collagen may affect the Alport GBM structure and function differently than α1α2(IV) collagen alone. For instance, the α5(IV) chain is a shared component of normal α3α4α5(IV) and “alternative” (α5)α6(IV) protomers, which may engage common cell-surface receptors, thereby signaling a “normal” GBM to adjacent podocytes or glomerular endothelial cells. Alternatively, the presence of α5α6(IV) collagen in tissues that undergo stretching, such as skin or smooth muscle (28),
suggests that this network may have evolved to withstand elastic tension. If so, then perhaps α5α6(IV) collagen may better withstand the high mechanical stress in the GBM than the α1α2(IV) network. This study of Col4a3−/− mice provides a foundation for future work to test these specific hypotheses.

As a first step toward elucidating the role of α5α6(IV) collagen and its potential effects on the Alport renal phenotype, we showed that abundant deposition of α5α6(IV) collagen in the Alport GBM was associated with longer renal survival in F1(B6 × 129) Col4a3−/− mice. Although B6 and 129 Col4a3−/− mice have distinct GBM composition and progress to renal failure at very different rates (mean ages to ESRD of approximately 194 and 66 d, respectively), a direct comparison between pure strains must take into account modifier loci on chromosomes 9 and 16, previously shown to be associated with slower progression in B6 Col4a3−/− mice (20). However, the confounding effect of these autosomal modifier loci may be avoided by comparing renal survival in (129 × B6) versus (B6 × 129) F1 male mice, which also deposit different amounts of α5α6(IV) collagen in the GBM. This analysis revealed that high GBM levels of α5α6(IV) collagen were associated with 46% longer renal survival. The mean difference was approximately 34 d, accounting for approximately 27% of the overall difference in renal survival between B6 and 129 Col4a3−/− mice. A similar estimate is obtained by comparing renal survival in Col4a3−/− mice and Col4a5−/− mice (a model of X-linked Alport syndrome) on the same genetic background. In B6 Col4a5−/− mice, which cannot assemble an α5/α6(IV) collagen network, the median age to ESRD is 161 d (29), which is approximately 33 d earlier than in B6 Col4a3−/− mice (20). Although other factors, such as imprinting at autosomal loci, cannot be ruled out entirely, the most parsimonious explanation that is consistent with all experimental data is that one or more X-linked loci, possibly including Col4a6 itself, may affect renal survival in Col4a3−/− mice by modulating the amount of α5/α6(IV) collagen that is deposited in the Alport GBM.

Ectopic α5α6(IV) collagen in the GBM that substitutes for the loss of α3α4α5(IV) collagen may be a general mechanism in mammals. This alternative isoform switch also has been reported in a naturally occurring canine model of autosomal recessive Alport syndrome (30), but the underlying mechanisms and the functional significance could not be addressed adequately using outbred dogs. An important, clinically relevant question is whether the same isoform switch occurs in human disease. If so, then how does it affect the renal Alport phenotype? Although α6(IV) collagen expression is not investigated routinely in Alport diagnostic biopsies, two studies have reported the absence of α5(IV) and α6(IV) collagen in the GBM of patients with autosomal recessive Alport syndrome (12,13). Nevertheless, ectopic expression of α6(IV) collagen in the human GBM was documented previously in one patient with Alport syndrome (31). Given the genetic diversity of the human population, it is possible that the alternative switch to α5/α6(IV) collagen may occur in a subset of patients with autosomal recessive Alport syndrome. A deeper understanding of the mechanisms that regulate the expression and the assembly of this network eventually may lead to new clinical applications. For instance, therapeutically induced GBM deposition of α5/α6(IV) collagen in patients with autosomal recessive Alport syndrome, perhaps via pharmacologic upregulation of COL4A6, may provide a novel, hitherto unforeseen strategy for preserving renal function and delaying the progression to renal failure in this disease.

Acknowledgments

This work was supported by grant 5P01 DK65123 (to D.B.B. and D.R.A.) from the National Institutes of Health and by the 2003 Carl Gottschalk Research Scholar Award from the American Society of Nephrology (to D.B.B.). J.H.M. is an Established Investigator of the American Heart Association.

We thank Parvin Todd, Selene Colon, and Patricia St. John for expert technical help. We are grateful to Dr. Brendan Lee for providing kidneys from Lmx1b mutant mice for analysis. We thank Dr. Ray Mernaug and Dr. He-ping Yan for their help in producing mAb 801.

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