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GENETIC DISORDERS – DEVELOPMENT

Novel collagen glomerulopathy in a homotrimeric type I collagen mouse (*oim*)

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Background: Oim/oim mice [osteogenesis imperfecta model; homozygous null for the proα2(I) collagen gene] synthesize exclusively the homotrimeric type I collagen isotype, $\alpha1(I)_3$, and are unable to synthesize the normal heterotrimeric type I collagen isotype, $\alpha1(I)_2\alpha2(I)$. Previous studies of the *oim/oim* mouse have focused on the musculoskeletal system, with no systematic evaluation of other organ systems.

Methods: Multiple tissues from *oim/oim*, *oim*/+ (heterozygous) and +/+ (wild-type) mice were examined for gross and histological abnormalities. Tissues were stained with (*1*) hematoxylin and eosin (to assess lesion formation), (*2*) picrosirius red (collagen content), and (*3*) periodic acid methenamine silver (basement membrane). Kidneys were further evaluated ultrastructurally by electron microscopy and immunohistochemically with anti-α1(I) and anti-α1(III) collagen antibodies.

Results: Histological analyses revealed accumulations of picrosirius red-positive material, consistent with collagen, in glomeruli of 28/29 *oim/oim* mice, with no evidence of mesangial cell proliferation. Only the most severely affected animals had evidence of increased capillary basement membrane thickening or mild inflammation around the affected glomeruli. Electron microscopy confirmed the presence of fibrillar collagen. Immunohistochemistry with anti-α1(I) collagen antibodies confirmed accumulation of type I collagen in the *oim/oim* glomeruli. The +/+ and *oim*/+ kidneys had normal mesangium with no evidence of infiltration of collagenous material.

Conclusions. This study demonstrates the first evidence, to our knowledge, of abnormal glomerular collagen deposition associated with a type I collagen defect. Further in vivo and in vitro studies are necessary to elucidate the mechanistic, functional, and pathological significance of the *oim/oim* collagen glomerulopathy.

Progressive accumulation of collagen in the glomerular mesangial matrix is a major factor in chronic renal disease, culminating in glomerulosclerosis and renal failure [1–3]. Excess extracellular matrix (ECM) production has been hypothesized to result from over compensation of the glomerular wound healing response [4, 5]. Though much is known about the progression of kidney fibrosis and glomerulosclerosis, very little is known mechanistically about the role of the ECM in the pathogenesis of the mesangial cell response. We present in this report the identification and characterization of a novel type I collagen glomerulopathy in the *oim* mouse.

Type I collagen is the predominant structural protein in many tissues, and abnormalities in type I collagen synthesis and structure are associated with many inherited and acquired connective tissue disorders [6–10]. The predominant isotype of type I collagen present in tissue is the heterotrimeric molecule, composed of two α1(I) chains and one similar, but genetically distinct α2(I) chain [6, 11]. The homotrimeric isotype consisting of three α1(I) chains, $\alpha1(I)_3$, has been shown to naturally occur in low levels in normal adult skin, during embryonic development, and during wound healing [11–14]. The α1(I) and α2(I) collagen chains are synthesized as precursor procollagen chains, which assemble into mature collagen fibrils after a complex series of co- and post-translational processing steps [6, 11]. Specific domains in the α2(I) and α1(I) chains have been identified that regulate fibril assembly [15–18].

In the normal glomerulus of the kidney there are three cell types: mesangial, epithelial, and tubular cells [5]. Mesangial cells compose 30 to 40% of the total glomerular cells. The kidney normally has very little type I or type III collagen, and its presence is generally associated with severe disease states [19–21]. In contrast to the intact kidney, cultured mesangial cells have been shown to produce type I and lesser amounts of types III, IV and V collagens [4, 5, 22, 23]. For this reason mesangial cell cultures are thought to mimic the collagen phenotype of diseased glomeruli.

The *oim/oim* (osteogenesis imperfecta model) mice are homozygous for a null proα2(I) collagen gene, *Cola2*,

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and are unable to synthesize functional proα2(I) collagen chains [24, 25]. Thus, in the absence of proα2(I)collagen chains the *oim/oim* synthesize exclusively the homotrimeric isotype of type I collagen, $\alpha1(I)_3$. The *oim/oim* mice are phenotypically similar to humans with moderately severe osteogenesis imperfecta (OI), characterized by severe skeletal fragility and deformity.

Previous studies of the *oim/oim* mouse have focused on the musculoskeletal system [26–34], but no systematic evaluation of other organ systems has been performed. In this study, multiple tissues from *oim/oim*, *oim*/+ (heterozygous) and +/+ (wild-type) mice were examined for gross and histological abnormalities. These examinations revealed a previously unrecognized renal abnormality in the *oim/oim* mice. Presented here are the histological, ultrastructural, and immunohistochemical features of a novel type I collagen glomerulopathy in the *oim/oim* mice.

METHODS

Animals

Homozygous B6C3Fe-*a/a-Cola2*$^{oim/oim}$ (*oim/oim*), heterozygous (*oim*/+) and wild-type (+/+) mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) [24]. All animals were housed in an AAALAC accredited facility, provided water and food (standard laboratory diet consisting of autoclavable rodent laboratory chow, 5010; Purina Mills Inc., Richmond, IN, USA) ad libitum, and handled according to an approved University of Missouri Animal Care and Use protocol. *Oim/oim, oim/*+ and +/+ genotypes were confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis [31].

Histology

As a pilot study, seventeen different tissues (including, heart, liver, lung, kidney, spleen, intestine, and brain) from six *oim/oim*, one *oim*/+ (heterozygous) and two +/+ (wild-type) mice were examined for gross and histological abnormalities. These examinations revealed a previously unrecognized renal abnormality in the *oim/oim* mice described herein. Kidneys were subsequently collected for histologic examination from an additional 23 *oim/oim* (29 total), 14 *oim*/+ (15 total) and 16 +/+ (18 total) mice. Female mice were euthanized and perfused (112 mm Hg, a pressure that mimics the normal *oim/oim* mouse systolic pressure) via the left ventricle or the abdominal aorta with 0.9% NaCl, followed by neutral buffered 10% formalin for histological evaluations. Multiple ages of mice, ranging from 3 to 25 months, were examined (Table 1). All kidneys were sectioned longitudinally and processed routinely for histologic examination. Five-micrometer sections were stained with hematoxylin and eosin (H&E) and examined for lesions.

Table 1. Age of mice examined for histologic lesions of the kidney

	Number of animals examined		
Age	*oim/oim*	*oim/*+	+/+
3–4 months	13	3	0
4–5 months	7	4	5
5–6 months	3	4	5
6–12 months	5	2	7
>12 months	1[a]	2[b]	1[c]

[a] *oim/oim* mouse was 25-months-old
[b] *oim*/+ mice were 25- and 30-months-old
[c] +/+ mouse was 13-months-old

Sections from select kidneys also were stained with picrosirius red to assess collagen deposition, periodic acid Schiff or periodic acid methenamine silver to assess basement membrane morphology, and alcian blue to assess proteoglycan content. Picrosirius red-stained sections were examined by conventional light microscopy as well as with polarized light. Glomerular lesions scores were determined from H&E-stained sections using the following scale: 0 = no lesions; 1= mild lesions, less than 50% of glomeruli affected; 2 = moderate lesions, less than 50% of glomeruli affected; 3 = moderate lesions, more than 50% glomeruli affected; 4 = severe lesions, more than 50% of glomeruli affected.

Morphometry

To estimate the amount of glomerular collagen deposition, picrosirius red-stained kidneys from 19 *oim/oim* mice were examined under polarized light and three adjacent ×200 views of the renal cortex were selected for imaging. Images were captured with an Olympus BX-60 digital camera, converted to tiff images in Adobe® Photoshop® 5.5 (Adobe Systems Incorporated) and analyzed using the Scion Image for Windows program (Scion Corporation, Frederick, MD, USA). Perivascular regions were deleted and total area occupied by collagen was calculated using the threshold mode. Glomerular collagen deposition was estimated by dividing collagen area by the number of glomeruli in that field.

Electron microscopy

Mice (*oim/oim*, $N = 2$; +/+, $N = 2$) were euthanized and kidneys were perfused with 0.9% NaCl (2 min), followed by neutral 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer (3 min). Sections were prepared as described [35].

Immunohistochemistry for α1(I) and α1(III) collagen

To determine if the fibrillar collagens, type I and type III were present, kidneys were evaluated by immunohistochemistry of paraffin-embedded sections. Heat-induced epitope retrieval in 0.1 mol/L citrate buffer was performed in a vegetable steamer. Endogenous peroxidase

was removed by treating slides with 3% hydrogen peroxide and non-specific antibody binding was blocked with a 5% bovine serum albumin (BSA) solution. Immunohistochemistry was performed using rabbit polyclonal anti-collagen type I primary antibody (LF-67; provided by L.W. Fisher [36]) diluted 1:100 in 5% BSA, a rabbit anti-collagen type III primary antibody (a kind gift from G. Kostka) diluted 1:500, a biotinylated anti-rabbit IgG secondary antibody diluted 1:200, a horseradish peroxidase-labeled avidin conjugate, a 3,3′diaminobenzidine tetrahydrochloride (DAB) substrate and hematoxylin counterstain. Staining was performed on an automated immunostainer (NexES; Ventana Medical Systems, Inc., Tucson, AZ, USA).

Statistics

To measure the strength of association between age, lesion score and glomerular collagen deposition, the Spearman Rank Order Correlation test in the statistical software package, SigmaStat (SPSS, Inc., Chicago, IL, USA) was used.

RESULTS

Previous studies of the *oim* mouse by our laboratory and others have focused on the musculoskeletal system [26–34], and until now no systematic evaluation of other organ systems has been performed. To evaluate the effect of the presence of homotrimeric type I collagen [absence of proα2(I)collagen] we systematically examined multiple organ systems from *oim/oim* (homozygous), *oim*/+ (heterozygous), and +/+ (wild-type) mice. Seventeen different tissues were examined (heart, brain, liver, lung, kidney, spleen, stomach, duodenum, jejunum, ileum, cecum, colon, salivary glands, uterus, ovary, quadriceps muscle, and urinary bladder) from *oim/oim* ($N = 6$), *oim*/+ ($N = 1$), and +/+ ($N = 2$) mice for gross and histological abnormalities. Except for lesions in the *oim/oim* kidney, no significant gross or histological lesions were seen. Kidneys were subsequently collected for histologic examination from an additional 23 *oim/oim* (29 total), 14 *oim*/+ (15 total) and 16 +/+ (18 total) mice. Histological examination of *oim/oim* mouse kidneys revealed accumulation of homogenous material in the glomerular tufts. This material was determined to be collagen on picrosirius red-stained sections (Fig. 1). No evidence of increased collagen deposition was observed in other parts of *oim/oim* mouse kidneys including blood vessels, perivascular spaces and interstitium. Lesion severity was scored on the following scale: 0 = no lesions; 1 = mild lesions, less than 50% of glomeruli affected; 2 = moderate lesions, less than 50% of glomeruli affected; 3 = moderate lesions, more than 50% glomeruli affected; 4 = severe lesions, more than 50% of glomeruli affected. In severely affected glomeruli, additional findings included capillary wall thickening, capsular adhesion, hyperplasia of parietal epithelial cells and crescent formation, thickening of capsule basement membrane, periglomerular fibrosis and mononuclear cell inflammation. However, in mild-to-moderately affected glomeruli none of these findings were evident, suggesting that collagen deposition was the primary lesion. Twenty-eight of 29 *oim/oim* mice evaluated developed glomerular lesions with some degree of collagen deposition. The amount of glomerular collagen deposition also was estimated by morphometric analyses. The glomerular area occupied by collagen ranged from 2155 μm^2 in the *oim/oim* mouse with no lesions to 38,372 μm^2 in an *oim/oim* mouse with a lesion score of 4. As expected, there was a positive correlation ($r = 0.743$; $P < 0.01$) between lesions scores and glomerular collagen deposition (Fig. 2). Curiously, neither lesion scores (data not shown) nor collagen deposition increased significantly as mice aged (Fig. 3). Select kidneys were stained also with alcian blue (pH 1 and pH 2.5) to assess proteoglycan content; no difference was evident in the proteoglycan content (data not shown). No glomerular lesions were seen in *oim*/+ (not shown) or +/+ mouse kidneys (Fig. 1).

The presence of fibrillar collagen in the *oim/oim* mesangium was confirmed by electron microscopy (Fig. 4). Wild-type mouse kidneys had normal mesangium, with no evidence of infiltration. The *oim/oim* mesangium was expanded 2 to 3 times, with infiltration by moderately electron-dense granular and fibrillar material. Many of the deposits showed clear fibrillar organization with bundles of fibrils, demonstrating the typical cross striation pattern of organized collagen, which appears consistent with the striation patterns of *oim/oim* homotrimeric type I collagen previously reported [37]. The glomerular basement membranes in both *oim/oim* and +/+ kidneys were unremarkable and the epithelial foot processes were intact.

To determine if the fibrillar collagen was composed of type I collagen and/or type III collagen, kidneys were evaluated immunohistochemically. Serial sections of kidney from a +/+ mouse, an *oim/oim* mouse with a lesion score of 1 and an *oim/oim* mouse with a lesion score of 4 were treated with LF-67 polyclonal anti-proα1(I) collagen antibodies, polyclonal anti-proα1(III) collagen antibodies, or control serum. Polyclonal anti-proα1(I) collagen antibodies demonstrated that the accumulated collagen in the *oim/oim* glomeruli consisted of type I collagen (Fig. 5). There was no evidence of type III collagen in the glomeruli of either *oim/oim* and +/+ kidneys; positive perivascular collagen staining served as an internal positive control (not shown).

DISCUSSION

Previous investigations of the *oim/oim* mouse have focused primarily on the musculoskeletal system with no

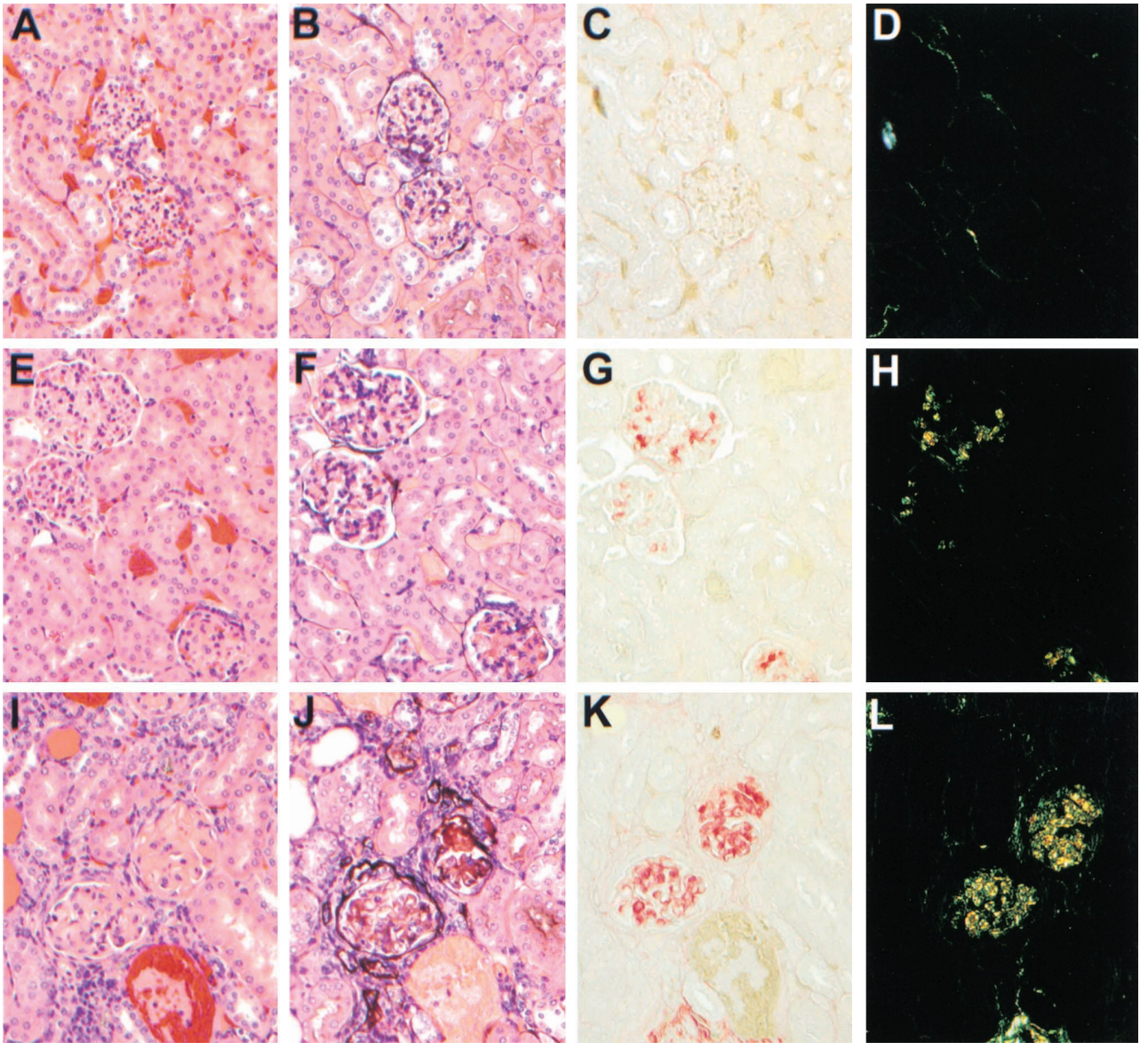
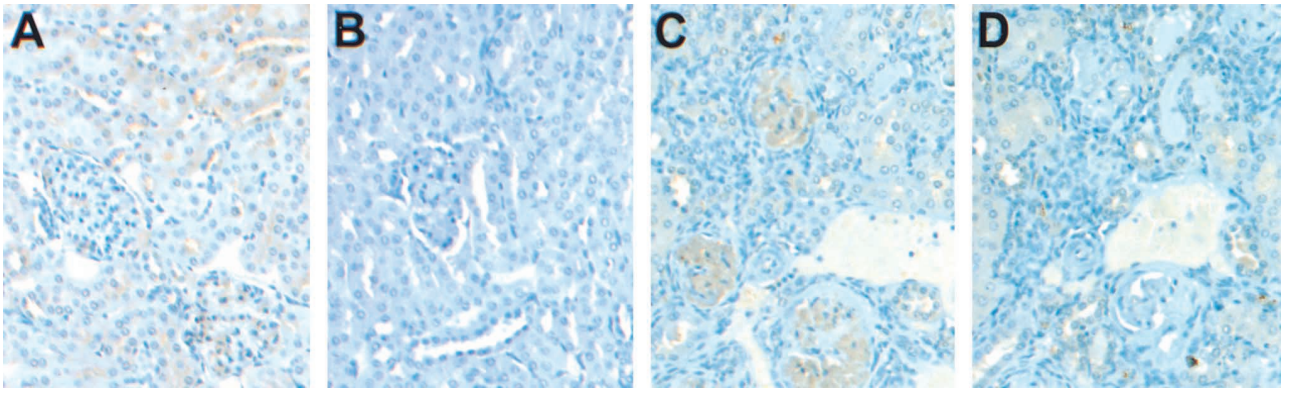


Fig. 1. Histological examination revealed accumulations of picrosirius red-positive material, consistent with collagen, in the glomeruli of *oim/oim* mice, which was not present in +/+ mouse kidneys. Histology of serial kidney sections of wild-type (+/+; *A, B, C, D*), mildly affected *oim/oim* (score of 1; *E, F, G, H*), and severely affected *oim/oim* (score of 4; *I, J, K, L*) mice stained with hematoxylin and eosin (A, E, I), periodic acid methenamine silver (B, F, J) and picrosirius red (C, G, K, non-polarized; D, H, L, polarized), at ×200 magnification. The +/+ mouse kidneys had normal mesangium, with no evidence of infiltration by abnormal material.



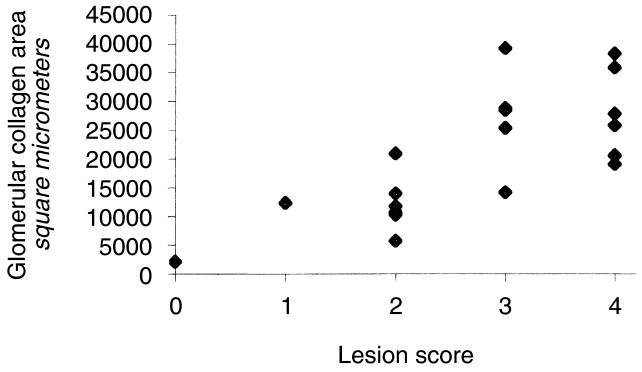


Fig. 2. Correlation between the amount of collagen deposited in glomeruli of the *oim/oim* mice and their lesion score. There was a positive correlation ($r = 0.743$) between these two variables (Spearman's rank correlation test; $P < 0.01$).

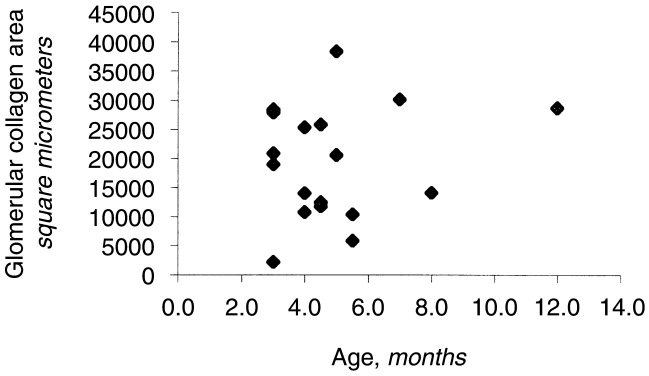


Fig. 3. Correlation between the amount of collagen deposited in *oim/oim* mice glomeruli and age of the mouse. There was no significant relationship between these two variables (Spearman's rank correlation test).

systematic evaluation of other organ systems. Our study demonstrates the first evidence, to our knowledge, of abnormal collagen accumulation in the glomeruli associated with a type I collagen defect. Furthermore, this report provides evidence that exclusive expression of homotrimeric type I collagen may be detrimental to kidney morphology.

Hereditary renal diseases are responsible for 16% of chronic renal failure in children [38]. Alport syndrome is the best known of the hereditary collagen defects, which results from defects in type IV collagen, a nonfibrillar collagen and a primary component of the basement membrane [39]. Direct association of kidney disorders with fibrillar collagen defects only recently has been reported with type III collagen, though the exact etiology for the over-expression of type III collagen is unknown [40–43]. This type III collagen glomerulopathy appears to be autosomal recessive, and is characterized by a diffuse increase in glomerular mesangial matrix and widening of capillary walls and in some cases progresses to renal failure [40–43]. Some patients develop hypertension, hemolytic anemia, and pulmonary disease as well. Electron microscopy confirmed fibrillar collagen was in the mesangial matrix and it was subsequently determined by immunohistochemistry to be primarily type III collagen and to a lesser extent type I collagen (localized primarily to the peripheral wall) [41, 43].

Though primary human collagen glomerulopathies have been described [39], it is the secondary collagen glomerulosclerosis and glomerular fibrosis that are much more prevalent and a common manifestation in end-stage renal disease, regardless of the origin [1–3, 5, 20, 44]. The predominant collagens associated with secondary collagen glomerulosclerosis and glomerular fibrosis are most often type IV collagens, then type III collagen, and generally only when it is very severe disease is type I collagen present [20, 44]. Yoshioka et al demonstrated in vivo that types III and I collagen often co-existed within nearly or totally sclerosed glomeruli, while type III collagen without type I collagen was present in moderately severe glomeruli [20].

Normally, the in vivo kidney has very little if any type I or type III collagen present, and its presence is generally associated with disease states [19–21]. In contrast, collagen expression by cultured mesangial cells (in vitro) is very different. Because mesangial cells in culture produce collagen, mainly type I, and to a lesser amount types III, IV, and V collagens, they are hypothesized to mimic the collagen phenotype of diseased glomerulus [4, 22].

It is controversial as to whether the type I collagen present in vitro or in vivo is homotrimeric or heterotrimeric type I collagen. Studies by Haralson, Jacobson and Hoover have demonstrated synthesis of both homotrimeric and heterotrimeric isotypes of type I collagen in cultured (in vitro) normal rat glomerular mesangial cells [4], whereas Ohyama et al reported only finding the heterotrimeric isotype [22]. Haralson et al's findings suggest that only half of newly synthesized collagen is released into the media, and the predominant type I collagen isotype in the cell layer was homotrimeric type I collagen. Ohyama et al also found type I collagen in the

Fig. 5. Immunohistochemistry using polyclonal anti-proα1(I) collagen antibodies (LF-67) demonstrate the accumulated collagen in the *oim/oim* mesangium consists of type I collagen. Serial kidney sections of a wild-type mouse (*A, B*) and an *oim/oim* mouse with a lesion score 4 (*C, D*) were treated with LF-67 polyclonal anti-collagen type I antibodies (A, C) or control serum (B, D), at ×200 magnification. Specific collagen staining was evident in glomeruli of *oim/oim* mice.

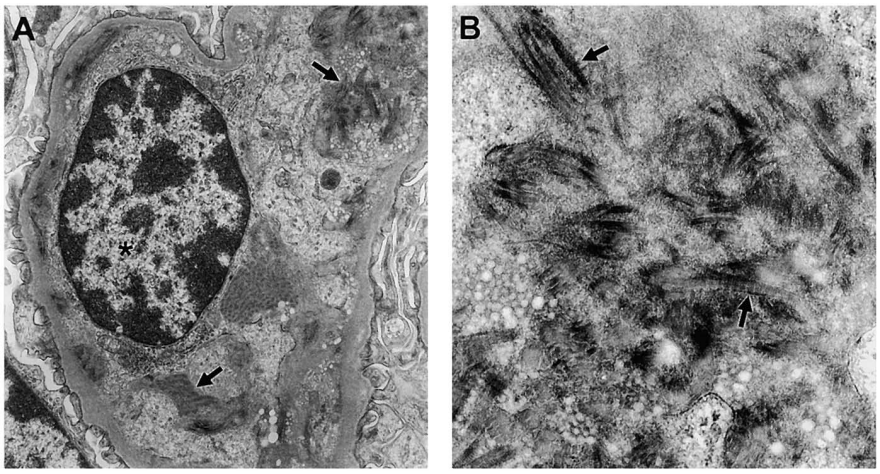


Fig. 4. Electron microscopy confirmed the presence of fibrillar collagen. The mesangium of the *oim/oim* glomeruli was expanded by 2 to 3 times, with infiltration by moderately electron dense granular and fibrillar material, which exhibited typical cross-striation of organized collagen. (*A*) Magnification (×9600) of an *oim/oim* glomerular mesangial cell (*indicates nucleus) demonstrating extracellular deposits with clear fibrillar organization and bundles of fibrils (arrows). (*B*) Magnification (×18,700) of a fibrillar deposit demonstrates typical cross striation of organized collagen (arrows). The epithelial foot processes were intact for both *oim/oim* and wild-type mice.

cultured mesangial cell layer, however, it was only the heterotrimeric isotype. These conflicting reports may be attributed to variations in the culturing conditions or possibly to mesangial cells, which may have dedifferentiated to the extent to result in reduced α2(I) expression. The accumulation of type I collagen in *oim/oim* mouse glomeruli suggest that homotrimeric type I collagen is under different regulatory control mechanisms than heterotrimeric type I collagen, and may provide a unique opportunity to investigate mechanisms of kidney damage and fibrosis.

Though the pathological significance of accumulated homotrimeric type I collagen in the mesangium of the glomerulus of *oim/oim* mice remains to be determined, it is perhaps analogous to the isotype alterations seen in Alport syndrome [39, 45, 46]. Alport syndrome, a hereditary disorder characterized by progressive nephropathy, results from defects in either proα3(IV), proα4(IV) or proα5(IV) collagen genes [39]. The normal adult glomerular basement membrane is composed of type IV collagen networks of predominantly the α3(IV)α4(IV)α5(IV) type IV collagen isotype, and the $\alpha1(IV)_2\alpha2(IV)$ type IV collagen isotype [39, 45, 46]. In the glomerular basement membranes of Alport syndrome patients the α3(IV)α4(IV)α5(IV) isotype is severely reduced or absent. The altered composition of their glomerular basement membranes is hypothesized to reflect failure of an early developmental switch; the fetal α1 and α2 chains persist in the adult glomerular basement membranes and are not replaced by α3, α4 and α5 chains [45, 46].

Accumulation of extracellular matrix can occur as a result of increased synthesis, decreased degradation, or some combination of the two. During the synthesis of type I collagen the amino- and carboxy-propeptide domains are cleaved from the procollagens prior to the mature collagen molecule assembling into collagen fibrils. Both amino- and carboxy-propeptides have been shown to have feedback inhibitory effects on collagen synthesis [15–17]. The carboxy-propeptide pre-translationally regulates collagen synthesis by internalizing and going into the nuclear compartment to down-regulate procollagen gene transcription [16]. Prockop and Fertala demonstrated that a nine amino acid peptide from the α2(I) collagen telopeptide (end of the triple helix and retained by the mature collagen) was sufficient to inhibit collagen fibril assembly [18], supporting the hypothesis that α2(I) collagen may have regulatory role in normal fibrillar formation and assembly. The major physiological regulators of ECM degradation in the glomerulus are matrix metalloproteinases (MMP) [47]. High rates of ECM turnover are characterized by renal disease and during normal renal development. Perhaps the metalloproteinases differentially regulate and/or have differential specificity to homo- and heterotrimeric type I collagen. Future studies are necessary to determine the pathogenesis of collagen deposition in the *oim/oim* mouse.

Transgenic mouse models may provide clues to the mechanisms involved in this collagen glomerulopathy [48–53]. Chatziantoniou et al describe the treatment of transgenic mice harboring the luciferase gene under the control of the murine proα2(I)collagen promoter with the nitric oxide synthesis inhibitor L-NAME (N^G-nitro-L-arginine-methyl ester), which resulted in the synthesis and deposition of type I collagen in the afferent arterioles and glomeruli [48]. The fibrosis appeared to be in part mediated by endothelin-induced activation of type I collagen expression, and independent of the L-NAME induction of hypertension. Sanderson et al described the transforming growth factor-β (TGF-β) transgenic mouse (the TGF-β transgene was expressed only in the liver, driven by the murine albumin promoter and enhancer) [49]. Two of the mouse lines derived from this transgene

exhibited renal disease. Histologically they developed mesangial expansion and thickened capillary loops, suggesting that chronically elevated circulating levels of TGF-β induces progressive glomerulosclerosis [50]. In this model initial glomerular abnormalities included mesangial expansion and interposition when podocyte morphological features were intact, suggesting the glomerular mesangium is the major target of the circulating TGF-β. Immunostaining revealed increased type I and III collagens in the mesangium, glomerular capillary loops and interstitium, while type IV collagen was unchanged. The increased collagen expression was due to increased mRNA (started as early as one week of age, prior to the appearance of glomerulosclerosis), and there was evidence of increased matrix protein production and decreased matrix remodeling [51]. TGF-β is known to be a bifunctional growth regulator in mesangial cells, stimulating collagens type I, IV, and fibronectin expression [51, 52]. TGF-β also may regulate matrix turnover by increased expression of the tissue inhibitor of metalloproteinase-1 (TIMP-1) renal tissue inhibitor of metalloproteinase-1 (also known to inhibit all MMP family members) [51]. Francki et al demonstrated in cultured mesangial cells from the SPARC (secreted protein acidic and rich in cysteine)-null mouse that in the absence of SPARC there was a significant reduction in type I collagen mRNA and protein, which correlated with a significant reduction in TGF-β1 mRNA and secreted TGF-β1 protein [53]. Their findings suggest that in murine mesangial cells, SPARC is essential to type I collagen expression and regulates the production of type I collagen through its effects on TGF-β1. Whether SPARC, TGF-β1, MMPs or TIMPs are altered in the *oim/oim* mouse remains to be investigated.

Patients with osteogenesis imperfecta (OI) are generally not thought to have renal manifestations. Two studies concerning renal function in OI patients reported hypercalciuria in 36 to 38% of OI patients, but it was not associated with nephrocalcinosis or with compromised kidney function [54, 55]. When considering the *oim/oim* mouse as a potential model for OI it is very important to understand that, though there are clinical similarities between the *oim/oim* mouse and type III OI patients, the type I collagen defect in the *oim* mouse represents the rare autosomal recessive form of OI [24], in which there are as few as four known reported cases worldwide (with no reported extraskeletal information) [56, 57].

It is also unknown whether the type I glomerulopathy in the *oim/oim* mice is progressive and whether the *oim/oim* mice will eventually develop renal failure as a result of severe glomerulopathy with age. Lesion severity, determined by lesion scores and the amount of collagen deposition, did not increase with age and preliminary clinical chemistry analyses of *oim/oim* and +/+ mice blood samples obtained from a limited number of the animals in this study suggest that *oim/oim* mice with kidney scores of 3 or less do not yet exhibit significant changes in blood urea nitrogen (BUN) and creatinine levels. However, one of three *oim/oim* mice with the kidney score 4 (glomerular collagen area = 38,372 μm^2) from which clinical chemistry analytes were measured had a moderately elevated BUN (35 vs. mean BUN of 22 for 14 other *oim/oim* mice; creatinine was not elevated in this mouse; data not shown). Further studies are necessary to determine the pathological significance of the type I collagen accumulation in the glomeruli and whether it is progressive.

This model, to our knowledge, represents the first example of a specific type I collagen defect resulting in a renal disease. Further studies in vivo and in vitro are necessary to elucidate the mechanistic, functional, and pathological significance of the *oim/oim* collagen glomerulopathy. Knowledge of the mesangial cell collagen phenotype during normal and diseased (pathologic) conditions is essential for understanding the mechanisms involved in the progression of glomerulosclerosis and the role played by mesangial cells in this process. The *oim* mouse offers a superb model system not only for examining the tissue specific role of type I collagen and more specifically the functional necessity of the α2(I) chain, but also for evaluating the role of proα2(I)collagen in the regulation of collagen expression in the glomerular mesangial cell.

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