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LABORATORY INVESTIGATION

Enhanced expression of "muscle-specific" actin in glomerulonephritis

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Enhanced expression of "muscle-specific" actin in glomerulonephritis. Increased expression of "muscle-specific" actin can be correlated with mesangial cell injury and proliferation in the rat. We performed similar immunohistochemical studies using two monoclonal antibodies (MoAb) to "muscle-specific" actins (HHF-35, a MoAb to pan muscle actin and α-SM-1, a MoAb to α-smooth muscle actin) on methyl Carnoy's fixed human renal biopsies which demonstrated a wide variety of inflammatory, proliferative, and non-proliferative glomerular diseases. Most glomerular diseases were associated with increased "smooth-muscle" actin expression. Exceptions almost invariably were disease settings without prominent cellular proliferation. As in the rat, there was a correlation of induced actin expression with increased glomerular cell proliferation, as detected by staining with a MoAb to proliferating cell nuclear antigen (PCNA). Double immunolabeling studies with an antibody to the leukocyte common antigen showed the great majority of $PCNA^+$ proliferating cells to be intrinsic glomerular cells rather than infiltrating leukocytes. These studies demonstrate that phenotypic changes of mesangial cells occur in both human and experimental glomerulonephritis, and are identifable in fixed tissue sections. These studies also suggest that markers of mesangial cell injury or activation and proliferation, such as immunostaining of renal biopsies for "muscle-specific" actins, might be useful diagnostic and/or prognostic indicators in proliferative or sclerosing glomerular diseases.

Injury to the mesangium is central to many glomerular diseases, including some characterized by immune complex deposition (such as IgA nephropathy, lupus nephritis, and membranoproliferative glomerulonephritis), and some diseases not usually thought to have a prominent inflammatory cell component (such as amyloidosis, diabetic nephropathy, and some forms of focal and segmental glomerulosclerosis). It is well recognized that there is considerable heterogeneity in the natural history of the disease process in each of these categories, and likewise that in renal biopsies the morphological appearance of involved glomeruli may display a wide spectrum of changes reflecting increased synthesis of matrix, cell proliferation, and remodeling of the tuft architecture. Much of our understanding of these processes currently is derived from studies of mesangial cells in culture, where the ability of these cells to produce components of the extracellular matrix, bind and/or secrete cytokines and numerous other biologically active substances, contract, undergo phenotypic modulation, and proliferate may be studied under defined conditions [1–3]. Our understanding of these processes in vivo is much more limited, and specifically there has been little consistent identification of phenotypic changes in mesangial cells in human disease. One of the few studies which have addressed this issue has demonstrated an increase in myosin in glomeruli of patients with diabetic nephropathy and some forms of glomerulonephritis [4, 5].

Recent studies of rodent models of glomerulonephritis by our group have shown that injury that results in mesangial cell proliferation (anti-Thy 1 antibody, Habu snake venom, Concanavalin A/anti-Concanavalin A antibody models) also results in increased expression of "muscle-specific" actins (principally smooth muscle actin) by mesangial cells [6]. "Muscle-specific" actins are those isoforms of the intracellular microfilament actin whose expression is thought to be specific to distinct types of muscle cells (smooth muscle α-actin, smooth muscle γ-actin, striated muscle α-actin, and cardiac muscle α-actin) [7, 8]. We have demonstrated that this increased expression of smooth muscle-actins in some models of injury is tightly linked to glomerular cell proliferation by virtue of its temporal correlation and by showing that experimental maneuvers that block the cell proliferation will also prevent the upregulation of α-smooth muscle actin gene expression and protein production in the glomeruli [6]. Further, as yet preliminary, studies by our group using a protocol of systemic infusion of angiotensin II into rodents indicates that in other settings, such as with increased intraglomerular pressure, the enhanced expression of smooth muscle actin isoforms occurs and may be dissociated from concomitant mesangial cell proliferation (**Note added in proof**).

In this study, we have investigated the relevance of these observations to human disease by utilizing similar phenotypic analyses of human biopsy tissue. Our finding that modest basal expression of "muscle-specific" actins in mesangial cells may be found in uninjured, apparently normal kidneys is demonstrative of a close ontogenetic relationship between mesangial cells and smooth muscle cells. We show that these actins can usually be detected in the mesangium in any form of glomerular injury, and that markedly increased expression is common in such proliferative glomerulonephridities as diffuse proliferative lupus nephritis and acute glomerulonephritis. Finally, as suggested by our studies in rodents, this study demonstrates a correlation between mesangial smooth muscle α-actin expression and proliferation of cells within the glomerulus. Hence, it appears that enhanced expression of "muscle-specific" actins may be a

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Table 1. Glomerular expression of "muscle-specific" actins in disease

Diagnosis	Number of cases	Number of glomeruli	Distribution of actin score by cases					Distribution of actin score by no. glomeruli				
			0	1	2	3	4	0	1	2	3	4
Minimal change disease (MCD)	15	228	0	6	7	2	0	49	119	58	2	0
Focal and segmental glomerulosclerosis (FSG)	11	132	0	3	7	1	0	27	43	58	4	0
Mesangial proliferative glomerulopathy (non-immune complex)	7	95	1	2	1	1	2	21	32	20	13	9
Membranous gN (non-lupus)	3	73	0	1	1	0	1	31	30	8	3	1
IgA nephropathy (predominantly sclerosing)	4	34	0	2	2	0	0	12	14	8	0	0
IgA nephropathy (all other)	9	152	0	2	3	3	1	38	43	58	11	2
Membranoproliferative GN	3	17	0	0	1	2	0	2	7	6	2	0
Lobular GN	3	22	0	1	1	1	0	3	8	8	3	0
Amyloid	8	87	0	2	4	1	1	17	34	28	7	1
Diabetic nephropathy	13	128	0	5	4	1	3	19	23	50	17	19
Diffuse lupus nephritis (DPLGN)	9	89	0	1	1	4	3	4	14	18	40	13
Membranous lupus nephritis	3	63	0	1	0	2	0	15	19	24	5	0
Acute GN	5											
Post-infectious	3	14	0	1	0	1	1	1	2	3	4	4
Cryoglobulinemic	1	31	0	0	0	0	1	3	4	7	0	17
NOC	1	9	0	0	0	1	0	4	1	1	3	0
Crescentic GN	14		0	3	4	6	1					
Crescentic glomeruli		66						13	15	13	19	6
Non-crescentic glomeruli		125						40	29	26	29	1
Transplant rejection	9	161	0	2	3	4	0	72	23	40	26	0
Normals	12	893	3	4	5	0	0	321	295	277	0	0

Abbreviations are: GN, glomerulonephritis; NOC, not otherwise classifiable.

useful phenotypic marker of mesangial injury and/or activation in human disease.

Methods

Source of tissue

Normal human kidney tissue ($N = 9$) was obtained from kidneys surgically excised because of the presence of a localized neoplasm. Tissues utilized for this study were obtained from macroscopically normal portions of kidney located at some distance from the neoplastic process. Additional normal tissue was obtained from fresh cadaver kidneys unable to be utilized for transplantation ($N = 3$).

Diseased human kidney was studied from a consecutive series of renal biopsies obtained over a two year period (1983 to 1984 at the University of Washington). Biopsies were excluded from this study for the following reasons: (a) tissue submitted in inappropriate fixative; (2) insufficient tissue remaining after completion of diagnostic workup; (3) fewer than three glomeruli remaining in tissue sections available for immunohistochemical studies. Biopsies in which the only diagnosis obtainable was nonspecific nephrosclerosis or end-stage renal disease were not considered; likewise, because of difficulty in interpreting results, biopsies with more than one pathologic process present (such as diabetic nephropathy and post-infectious glomerulonephritis) were also excluded from this study. The remaining 116 cases with acceptable tissue were studied. These cases were obtained from patients with a broad range of renal diseases (Table 1).

There were two cases (one of minimal change disease, one of focal and segmental glomerulosclerosis) which only had evaluations for "muscle-specific" actins due to inability to achieve technically satisfactory PCNA immunostaining prior to exhaustion of the tissue.

Fixation

All tissues were fixed in methyl Carnoy's solution (60% methanol, 30% chloroform, 10% acetic acid) and processed and embedded in paraffin utilizing conventional techniques [9].

Immunohistochemistry

Sections of methyl Carnoy's fixed tissue were deparaffinized with Histoclear (National Diagnostics, Highland Park, New Jersey, USA) and graded ethanols, blocked with 0.3% hydrogen peroxide and 0.1% sodium azide, and washed with PBS (138 mM NaCl, 2.7 mM KCl, 3.2 mM Na_2HPO_4, 1.5 mM KH_2PO_4 pH 7.3) containing 0.1% BSA (Sigma Chemical Co., St. Louis, Missouri, USA) and 0.01% Triton X-100. The tissue was then incubated with one of the primary murine monoclonal antibodies (see below), and subsequently processed using a streptavidin-biotin immunoperoxidase method with 3,3′-diaminobenzidine (with nickel chloride enhancement) as the chromogen. Sections were counterstained with methyl green.

Antibodies

Three murine monoclonal anti-muscle antibodies were used for immunohistochemical evaluation. Anti-α-SM-1 [10], with specificity for the smooth muscle α-actin isoform (gift of G. Gabbiani) and HHF-35 [11], reactive with all four "muscle-specific" actin isoforms (smooth muscle α and γ, cardiac muscle α, and striated muscle α actin isoforms) have been well characterized previously. A murine monoclonal antibody to human desmin (DAKO Corp., Carpinteria, California, USA),

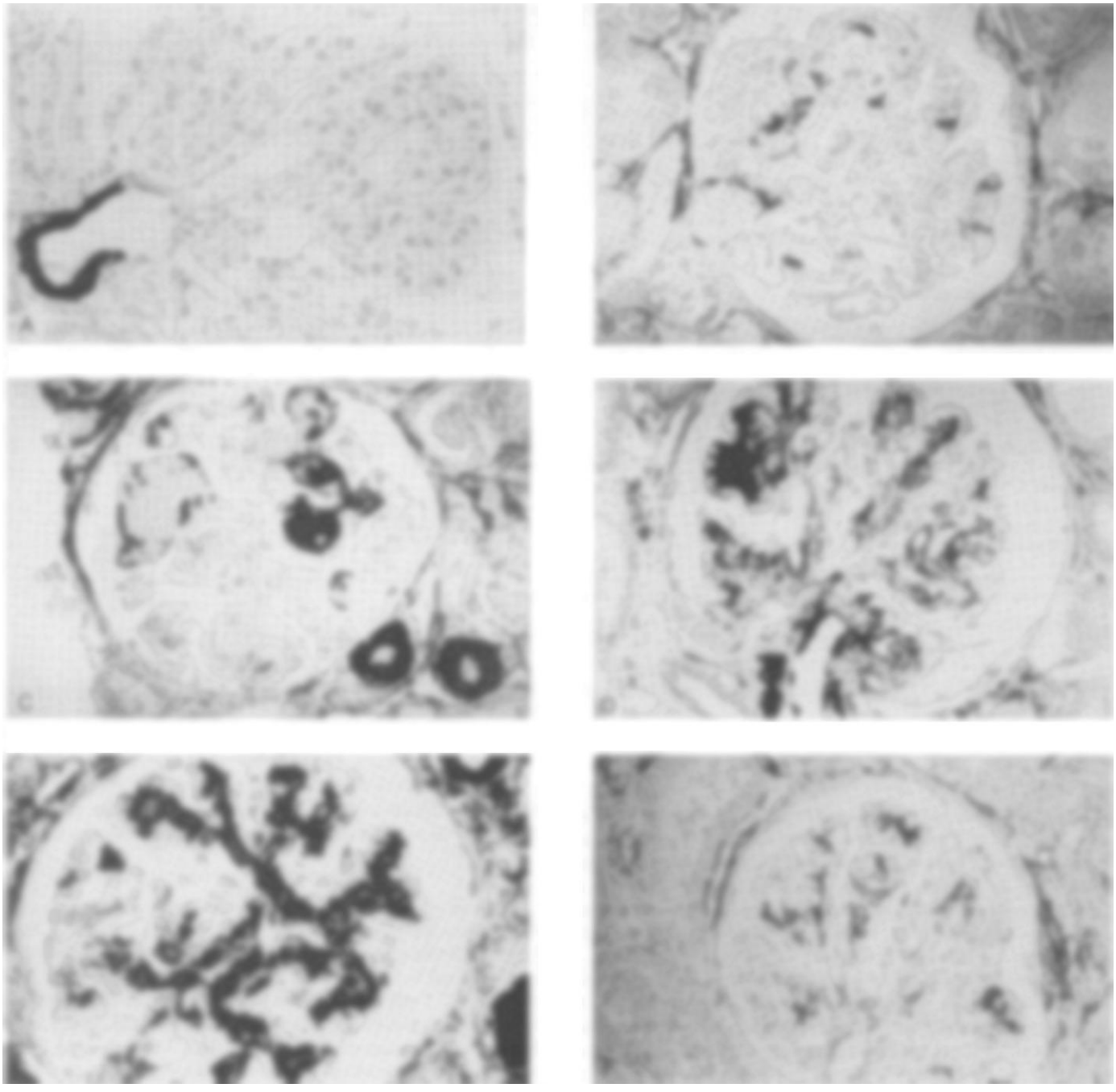

Fig. 1. *Expression of "muscle-specific" actin in normal and diseased glomeruli.* **A.** Minimal change disease. Detectable levels of α-smooth muscle actin are expressed by smooth muscle cells in a hilar arteriole, but not by cells of the glomerular tuft. 300×. **B.** Glomerulus obtained from apparently normal kidney resected for localized tumor. α-Smooth muscle actin scores ranged from 0 to 2 in these cases. This level of expression would be assigned a score of 2. 300×. **C.** AL-amyloidosis. α-Smooth muscle actin score 3. There is segmental staining of the glomerular tuft including cells surrounding a nodule of deposited amyloid. There is also staining of adjacent muscular arteries and periglomerular and interstitial mesenchymal cells. 300×. **D.** Diabetic glomerulosclerosis. α-Smooth muscle actin score 4. Prominent mesangial staining for actin is apparent, especially in areas of prominent, nodular, sclerosing injury. 300×. **E.** Mesangial proliferative glomerulopathy. α-Smooth muscle actin score 4. Same case as Figure 2B. Staining is predominantly located in the mesangium. 380×. **F.** Crescentic glomerulonephritis of non-immune complex origin (non-crescentic glomerulus). α-Smooth muscle actin score 3. The interstitium adjacent to Bowman's capsule and adjacent to the peritubular capillaries contains a population of spindled cells which also express α-smooth muscle actin. 250×.

an intermediate filament demonstrable in some populations of human smooth muscle cells, was also employed. PCNA/cyclin was detected with the murine IgM monoclonal antibody 19A2 (Coulter Corp., Hialeah, Florida, USA) [12]. For all biopsies a negative control consisted of substitution of the primary antibody with both irrelevant murine monoclonal antibodies and

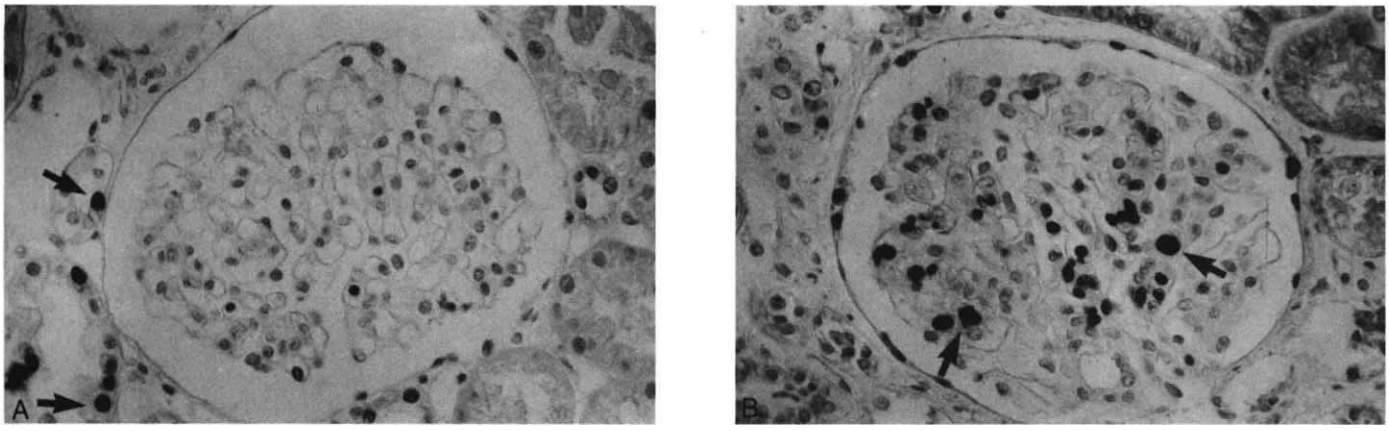

Fig. 2. *Glomerular cell proliferation detected by immunolabeling with an antibody to proliferating cell nuclear antigen (PCNA).* **A.** Minimal change disease. While there is extensive staining of adjacent tubular epithelial cell nuclei for PCNA (arrows), there is no staining of the glomerular tuft. Same case as shown in Figure 1A. 300×. **B.** Mesangial proliferative glomerulopathy. An example of marked cell proliferation detected by immunostaining for PCNA. Most positive cells (arrows) in the glomerular tuft are located in the mesangium. Same case as that shown in Figure 1E. 300×.

PBS. Positive internal controls in each biopsy consisted of actin-positive smooth muscle cells comprising the renal vasculature for the α-SM-1, HHF-35, and anti-desmin antibodies, and proliferating tubular epithelial cells for the PCNA/cyclin antibody.

Analysis of immunohistochemistry

Mesangial actin. Glomerular expression of "muscle-specific" actins was graded semiquantitatively according to the following scale:

0 = None
1 = Trace mesangial staining
2 = Weak, segmental mesangial staining; usually involving a small minority of glomeruli present
3 = Strong, segmental mesangial staining; usually involving a majority of glomeruli present
4 = Strong, diffuse mesangial staining; usually involving all glomeruli present

Glomerular cell proliferation. Proliferation was expressed as the number of cells per glomerular cross-section that were reactive with an antibody recognizing the proliferating cell nuclear antigen (PCNA)/cyclin as determined by immunocytochemistry. PCNA/cyclin is an auxiliary protein to DNA polymerase-δ; expression is restricted to late G_1, S, G_2, and M phase of the cell cycle [13].

Quantitation of $PCNA^+$ cells was performed by counting all positive cells within the glomerular tufts and dividing by the number of glomeruli present in each biopsy. All glomerular tufts were counted in each biopsy.

Double-immunolabeling immunohistochemistry

Double labeling for PCNA and leukocyte common antigen (LCA). Double immunolabeling of tissue sections was performed to evaluate if the proliferating glomerular cells detected by immunolabeling with the 19A2 antibody were of intrinsic glomerular origin or could be attributable to a population of infiltrating leukocytes.

Methyl Carnoy's fixed, paraffin embedded surgical biopsies from the fourteen cases with highest glomerular proliferation indices were sectioned and mounted on aminopropylmethoxysilane (APTS) coated slides. After deparaffinization and rehydration, the sections were incubated for five minutes with 3% hydrogen peroxide to inhibit endogenous peroxide reaction. They were then incubated with 10% normal goat serum for 30 minutes at room temperature (rt). Without rinsing the slides, the excess normal goat serum was removed and anti-PCNA antibody (Coulter Corp.), diluted at 4°C in a humid chamber. Following PBS washes, the sections were incubated with goat-anti-mouse IgM-gold (Auroprobe LM GAMIgM, Amersham, Arlington Heights, Illinois, USA) (1:40 dilution in PBS containing 1% BSA and 0.1% gelatin) for one hour at room temperature. Sections were washed and the gold was visualized with a silver enhancement reaction kit (IntenSE M, Amersham). The sections were then incubated sequentially with anti-CD45 (DAKO Crop.; 1:50 dilution, 60 min, rt), biotinylated horse anti-mouse IgG (Vector, Burlingame, California, USA; 1:200 dilution, 30 min, rt), and avidin-biotin-peroxidase complex (Vector; 30 min, rt). The peroxidase was developed with a diaminobenzidine substrate solution and then counterstained with methyl green. Negative controls included omitting either the PCNA or the CD45 antibody in the staining procedure.

Statistical analysis

Values are expressed as the mean ± SE. Comparison of actin and PCNA values between disease (all categories) and non-disease controls was performed using the Mann-Whitney U test. ANOVA was used for pairwise comparisons of group means, using the Tukey method to adjust for multiple comparisons. Correlation of actin and PCNA values was performed using the actual PCNA value plus a constant 0.5 transformed to a natural logarithmic scale, which reduced heteroscedasticity and identified a more linear relationship with actin values.

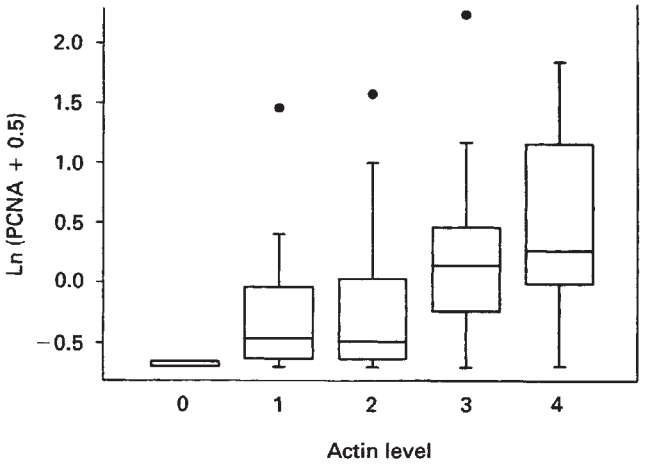

Fig. 3. *Boxplot graphic depiction of glomerular actin versus cell proliferation, indicated as the natural logarithm of the PCNA value plus a constant 0.5.* Areas within the boxes include those cases falling in the 2nd and 3rd quartile range of data obtained for PCNA. The lines within the box indicate the median PCNA value for each given actin value. The bars indicate those cases in the 1st and 4th quartiles of PCNA values, with the exception of three outliers which are represented by individual points in the graph.

Results

"Muscle-specific" actin expression in normal and diseased kidneys

The distribution of scores by individual cases for reactivity with the α-smooth muscle actin antibody in all cases utilized in this study is given in Table 1, with illustrations of typical glomerular actin expression patterns shown in Figure 1. Also given are the scores for each individual glomerulus examined within the given disease categories. The values obtained with the HHF-35 pan-muscle actin antibody were essentially similar, although in general staining appeared slightly more intense with the α-smooth muscle actin antibody. Because of the generally congruent staining with these two antibodies, and because of the narrower specificity of the α_1-smooth muscle actin antibody, it seems likely that much of the actin reactivity detected by HHF-35 is directed against the smooth muscle isoform recognized by the α-SM-1 antibody, although activity against the γ-smooth muscle actin isoform cannot be excluded. We therefore consider that immunoreactivity for either of the antibodies to "muscle-specific" actins in the glomerulus is in fact reactivity for "smooth muscle" actin. Descriptive statistical summary of the actin findings is given in Table 2.

Normal kidneys. While some normal kidneys were without detectable levels of glomerular α-smooth muscle actin expression, this was not always the case. As indicated in Table 1, most kidneys contained some glomeruli that showed focal, modest smooth muscle actin expression of 1^+-2^+ intensity in mesangial areas. The typical appearance of glomeruli expressing either none or modest smooth muscle actin expression is shown in Figures 1A and 1B. No normal kidney showed prominent actin expression (3^+-4^+). Positive controls in every case showed marked concomitant reactivity with both HHF-35 and α-SM-1 antibodies of the smooth muscle cells of the renal arterial tree. There was no detectable staining of glomeruli with the anti-desmin antibody in either normal or diseased kidneys. As in the case of muscle-specific actin, all cases showed prominent desmin expression by smooth muscle cells in the renal arterial vasculature.

Kidneys with glomerulopathies. Representative illustrations of α-smooth muscle actin expression in various glomerulopathies are presented in Figure 1. By histologic analysis, glomerular actin expression is virtually always mesangial in location regardless of disease type. Only in cases where the expression of this isoform is most intense, as illustrated in Figures 1D and 1E, is it not possible to exclude a component of concomitantly induced expression of this protein in glomerular endothelial cells or visceral epithelial cells. In general, the distribution and intensity of actin expression within glomeruli was closely correlated, that is, weak or modest actin expression (1^+-2^+ intensity) most often was seen in only one or two lobules of a glomerulus while more intense (3^+) staining typically involved more than half of the lobules present (Fig. 1C). Some variability in actin expression was seen in individual glomeruli within any given biopsy. In all cases, biopsies were assigned the highest actin score obtained by any of the glomeruli within them. While uniformity of expression was more readily seen in glomeruli from biopsies with high actin scores, our approach does not reflect the heterogeneity of expression that was seen in a number of the cases assigned lower actin scores (2 or less). This heterogeneity was frequent in some categories such as minimal change disease or normal kidneys where a positive actin score may be assigned on the basis of staining that is present in only a minority of the glomeruli within the biopsy, with most being unreactive with the anti-muscle-actin antibodies. The extent of heterogeneous expression of α-actin can be assessed by inspection of that portion of Table 1 in which the actin scores for all of the glomeruli within each disease category are given.

Although not a focus of this particular study, Figures 1B-E also illustrate that a population of interstitial cells, present between the peritubular microvasculature and tubular basement membranes, also commonly express the α-smooth muscle actin isoform.

The extent of smooth muscle actin expression in the various disease categories studied is given in Table 1. When actin expression in all glomerulopathies regardless of diagnosis was compared with basal actin expression detected in the normal controls (Table 2), statistically significant elevation of actin expression was demonstrable in the diseased group (Mann-Whitney test, $P = 0.015$).

Pairwise comparison of actin expression by disease category resulted in a statistically significant difference ($P < 0.05$) between mean actin levels in the normal and diffuse proliferative lupus nephritis groups. This is a finding of interest but not clearly established, as the statistical method of comparison requires assumptions about the distributions of the populations tested that are not strictly met in this study. In essence, as indicated in Tables 1 and 2, there is a trend for actin expression to be highest in those disease groups with the highest degree of cellular proliferation as normally detected histologically as well as by direct quantitation with the PCNA antibody as detailed below. But because of the considerable heterogeneity of actin expression within disease groups, the samples are not sufficiently large to achieve statistical significance.

Table 2. Statistical summary

Diagnosis	ACTIN				PCNA			
	N	Mean	SEM	Median	*N*	Mean	SEM	Median
1. Minimal change disease (MCD)	15	1.73	0.18	2	14	0.17	0.09	0.04
2. Focal and segmental glomerulosclerosis (FSG)	11	1.82	0.18	2	10	0.30	0.08	0.29
3. Mesangial proliferative glomerulopathy (non-immune complex)	7	2.14	0.56	2	7	1.22	0.73	0.50
4. Membranous GN (non-lupus)	3	2.33	0.88	2	3	0.08	0.05	0.07
5. IgA nephropathy (predominantly sclerosing)	5	1.20	0.38	1	5	0.22	0.08	0.14
6. IgA nephropathy (all other)	8	2.13	0.29	2	8	0.50	0.26	0.29
7. Membranoproliferative GN	3	2.67	0.33	3	3	0.41	0.30	0.24
8. Lobular GN	3	2.00	0.58	2	3	0.33	0.33	0.00
9. Amyloid	8	2.38	0.42	2	8	1.21	0.59	0.45
10. Diabetic nephropathy	13	2.15	0.34	2	13	0.93	0.68	0.80
11. Diffuse lupus nephritis (DPLGN)	9	3.00	0.33	3	9	1.12	0.61	0.50
12. Membranous lupus nephritis	3	2.33	0.66	3	3	0.53	0.28	0.67
13. Acute GN	5	3.00	0.55	3	5	1.95	0.37	1.92
14. Crescentic GN	14	2.36	0.25	2.5	14	0.81	0.13	0.73
15. Transplant rejection	9	2.22	0.28	2	9	1.12	0.46	0.50
16. Normals	12	1.33	0.30	1.5	12	0.06	0.01	0.05
Categories 1–15	116	2.19	0.10	2	114	0.76	0.12	0.33

Comparison of actin expression with markers of cellular proliferation (PCNA)

The typical appearance of glomeruli and adjacent tubulointerstitial tissue stained for PCNA is illustrated in Figure 2, illustrating absence of proliferation and prominent proliferative activity respectively. The mean cell proliferation indices by disease category are given in Table 2. All normal kidneys exhibited a cell proliferation index of <0.15 positive cells/glomerular cross section. When PCNA scores for all disease categories combined are compared with those of the normal groups, the differences are statistically significant (Mann-Whitney, $P < 0.004$).

Actin and log PCNA had a statistically significant correlation coefficient of 0.457 ($P < 0.001$, one sample *t*-test). The results, illustrated in the box plot graph of Figure 3, indicate that regardless of diagnosis, there is a general linear correlation between glomerular α-smooth muscle actin expression and glomerular cellular proliferation.

Accordingly, the highest cell proliferation index values were concentrated in those glomerulopathies with highest expression of α-smooth muscle actin and conventionally thought of as proliferative glomerulonephritis (acute glomerulonephritis, diffuse proliferative lupus nephritis, and membranoproliferative glomerulonephritis). The one disease category with surprisingly high PCNA expression was that of amyloidosis, a disease not usually thought to be characterized by cellular proliferation. In some cases of amyloidosis there was elevated glomerular actin expression as well.

Double immunolabeling studies

Double immunolabeling was performed with antibodies reactive with PCNA and with LCA in the fourteen cases having the highest glomerular proliferative indices as detected by staining with the anti-PCNA antibody alone. There was nearly complete separation between those glomerular cells expressing the leukocyte common antigen and $PCNA^+$ cells, with greater than 95% of $PCNA^+$ cells unreactive with the antibody to LCA. Of interest was the finding that in the interstitium, aggregates of infiltrating LCA expressing mononuclear inflammatory cells contained a small proportion of cells that were also reactive with the antibody to PCNA, indicative of intrarenal leukocyte proliferation.

Discussion

"Muscle-specific" actins are any of four isoforms of the intracellular microfilament actin (smooth muscle α-actin, smooth muscle γ-actin, striated muscle α-actin, cardiac muscle α-actin) [7, 8, 14]. These isoforms can be distinguished from the cytoplasmic β and γ actin isoforms, which are widely distributed in nucleated cells, by their mobility on electrophoretic gels and by differing epitopes which can be recognized by currently available monoclonal antibodies. It is generally accepted that cellular expression of "muscle-specific" actins implies cellular derivation from either smooth muscle, cardiac muscle, or skeletal muscle, depending on the actin isoform expressed. This is a useful construct with which to approach phenotypic studies of tissue injury, but we remain cautious in the unequivocal acceptance of this idea because examples of anomalous, low level expression of these actin isoforms have been observed in non-muscle derived cells both in vivo and in vitro [14–19]. Our use of quotation marks with the term muscle-specific is meant to reflect this element of uncertainty as to the absoluteness of the relationship between actin isoform expression and muscle derivation.

The findings of this study were established by using two distinct, well characterized antibodies specifically reactive with muscle actin isoforms. Both of these antibodies have been characterized by Western blotting, and both have been investigated in a variety of disease processes using the tissue immunocytochemical procedures employed in this study [10,

11, 14, 19]. Neither has ever been shown to be cross reactive with matrix proteins by either Western blot or tissue localization studies. Previous localization studies in rat models of mesangial injury by our group have shown cellular localization of the HHF-35 muscle actin specific antibody by immunoelectron microscopy [6], and demonstrated that immunohistochemical detection of increased actin expression is correlated with induction of actin mRNA by Northern analysis. Finally, preliminary observations on human kidney using immunoelectron microscopy has provided further support for the conclusion that the extraglomerular renal interstitial reactivity seen with both of these antibodies is confined to a population of interstitial cells (Alpers CE et al, manuscript in preparation).

A major finding in this study is the demonstration that "muscle-specific" actins, and specifically α-smooth muscle actin, can usually be detected in the mesangium in any form of glomerular injury. These actins are less regularly but still frequently present in the mesangium of histologically normal kidneys obtained from patients without known renal disease other than localized tumors. In the case of normal kidneys, expression of "muscle-specific" actins, when present, is often focal and sparse, and is never associated with a grade higher than 2. It is not known if those normal kidneys with α-smooth muscle actin expression are truly "normal"; that is, if these kidneys may have come from patients with minor or unrecognized conditions such as mild hypertension or other disease processes. Nonetheless, this finding does provide additional evidence for the postulate that mesangial cells may have a close ontogenetic relationship with pericytes and/or smooth muscle cells, cell types which characteristically express the α-smooth muscle actin isoform [7, 8, 20].

Glomerular mesangial cells, in normal or diseased kidneys, were uniformly without detectable expression of desmin. In agreement with these results, studies by two groups of investigators also could not demonstrate the presence of desmin in normal or diseased human mesangium [21, 22]. However, one other group has localized desmin to normal human mesangium with an immunofluorescence technique [23]. Differences in sensitivity or dilutions of the antibodies employed may account for these differences, and the possibility that mesangial cells express low levels of desmin not readily detectable by tissue immunohistochemistry cannot be excluded. The significance of our findings is unknown, but studies by a number of others have shown that desmin expression in both a relatively specific and a relatively insensitive marker of smooth muscle cells [24, 25]. The present findings are in keeping with a concept of the mesangial cell as smooth muscle cell-like, but still maintaining its own distinctive phenotypic features.

In contrast to normal kidneys, enhanced expression of "muscle-specific" actins, and specifically α-smooth muscle actin, both in intensity and distribution, is a sensitive marker of mesangial injury detectable in fixed tissue sections. As seen in Table 1, enhanced expression may be seen in any form of glomerular injury, and markedly increased expression is common in proliferative glomerulonephritis such as diffuse proliferative lupus glomerulonephritis and acute glomerulonephritis. This finding closely follows that obtained in studies of immune complex mediated mesangial injury in the rat, where marked expression of α-smooth muscle actin by mesangial cells correlates with the onset of proliferation [6]. Alternatively, we found a few cases of diffuse glomerulonephritis as determined by diffuse patterns of immune complex deposition (such as lupus nephritis) which had low levels of α-smooth muscle actin expression. Our review of such cases showed that they were noteworthy even at the time of initial diagnosis because they were almost always associated with little cellular proliferation, a finding then thought discrepant with the extent of immune complex deposition that was demonstrable by immunofluorescence and electron microscopy. As discussed below, these findings in aggregate suggest that in general there is a strong association between α-smooth muscle actin expression and cellular proliferation in the glomerulus.

An unexpected finding of interest was that pronounced expression (that is, grade 3^+ or higher) of α-smooth muscle actin may be seen in mesangiopathic conditions not previously associated with cellular proliferation such as amyloidosis and nodular diabetic glomerulosclerosis. The finding of high actin expression in some cases of diabetes, which were without other evidence of superimposed inflammatory injury, is indicative of an altered physiologic state of mesangial cells in this setting. This finding in human biopsies is of particular interest in view of recent observations of Floege et al, which link induced mesangial cell α-actin expression to that time period of induced synthesis of matrix components in the anti-Thy 1 model of mesangial injury [26]. Occasionally, the pronounced mesangial actin expression extended to other diseases not usually thought of as mesangiopathic, such as minimal change disease. Because we still do not know the conditions which govern expression of actin isoforms by mesangial cells, it is not possible to explain how such diverse conditions such as an acute glomerulonephritis with cellular proliferation and influx of inflammatory cells and a slowly progressive process of nodular mesangial sclerosis may each result in apparently equivalent levels of α-smooth muscle actin. Therefore, our observations indicate that enhanced α-smooth muscle actin expression in human glomeruli may be best interpreted at the present time as indicative of mesangial cell perturbation or activation.

The second major finding in the present study, which also is consistent with prior studies in the rat, is that there is a correlation between high α-smooth muscle actin expression and glomerular cell proliferation, as detected by reactivity of glomerular cells with an antibody to PCNA [6]. This finding is somewhat remarkable in that unlike the controlled experimental situation in the rat, where conditions and the timing of biopsies are controlled and hence impart a certain uniformity to morphologic injury, our study of human biopsies necessarily includes lesions within diagnostic categories at all stages of initiation and progression. This feature probably accounts in part for the variability of proliferative indices obtained between individual cases within specific disease groups. Although shown in several studies to be a good marker of cellular proliferation [12, 13, 27–30] one potential caveat with PCNA staining is that it could conceivably react with cells that have entered the cell cycle but undergone subsequent arrest of their progression through cell division. In this scenario, expression of PCNA could at times be an additional marker of cell activation without necessarily indicating cell division.

The significance of our findings is not yet known. It is

tempting to speculate that increased levels of α-smooth muscle actin expression and acquisition of a more muscle-like phenotype by mesangial cells in disease states may affect mesangial cell contractility and hence contribute to the altered hemodynamics encountered in glomerular injury [31]. The studies of Floege et al noted above suggest the possibility that finding prominent α-smooth muscle actin expression in some human biopsies may reflect active production by mesangial cells of matrix, which may be reflected in the chronic sclerosing lesions encountered in amyloidosis and diabetic nephropathy. While it would be premature to identify enhanced expression of α-smooth muscle actin as a marker of active mesangial remodeling or scarring, histologic identification of such markers may have eventual clinical utility. For example, recent experimental investigations in the rat suggest that diseases associated with mesangial cell matrix production may eventually be targeted for therapeutic interventions such as administration of anti-transforming growth factor-β antibody [32], and it is to be hoped that such therapies may one day be employed in appropriately identified cases of human disease.

It is also possible that enhanced expression of α-smooth muscle actin, or other as yet unidentified markers of mesangial injury or activation, may have prognostic significance in specific disease states. Obvious examples where a potential association might be found are in the identification of those subsets of patients with minimal change disease who subsequently develop focal and segmental glomerulosclerosis, and those patients with IgA nephropathy who develop glomerulosclerosis. Studies to evaluate such possibilities are currently being pursued.

In conclusion, these studies demonstrate that enhanced expression of "muscle-specific" actins is a sensitive, albeit non-specific, marker of mesangial injury detectable in fixed tissue sections of human renal biopsies. In many cases, this enhanced expression also is a marker for cellular proliferation within glomeruli. We believe that identification of this expression in renal biopsies, as well as potential additional markers of altered phenotype of glomerular cells, may eventually prove to have diagnostic or prognostic significance in the assessment of human glomerular diseases.

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Note added in proof

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References

1. LOVETT DH, STERZEL RB: Cell culture approaches to the analysis of glomerular inflammation. *Kidney Int* 30:246–254, 1986
2. SCHLONDORFF D: The glomerular mesangial cell: An expanding role for a specialized pericyte. *FASEB J* 1:272–281, 1987
3. KRIESBERG JI: Cell biology and biochemistry of the glomerular mesangium. *Miner Electrol Metab* 14:167–175, 1988
4. SCHEINMAN JI, FISH AJ, MICHAEL AF: The immunohistopathology of glomerular antigens: The glomerular basement membrane, collagen, and actomyosin antigens in normal and diseased kidneys. *J Clin Invest* 54:1144–1154, 1974
5. SCHEINMAN JI, FISH AJ, MATAS AJ, MICHAEL AF: The immunohistopathology of glomerular antigens II. The glomerular basement membrane, actomyosin, and fibroblast surface antigens in normal, diseased, and transplanted human kidneys. *Am J Pathol* 90:71–88, 1978
6. JOHNSON RJ, IIDA H, ALPERS CE, MAJESKY MW, SCHWARTZ SM, PRITZL P, GORDON K, GOWN AM: Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis: α-smooth muscle actin is a marker of mesangial cell proliferation. *J Clin Invest* 87:847–858, 1991
7. VANDEKERCKHOVE J, WEBER K: At least six different actins are expressed in higher mammal: An analysis based on the amino acid sequence of the amino-terminal tryptic peptide. *J Mol Biol* 126:783–802, 1978
8. SKALLI O, VANDEKERCKHOVE J, GABBIANI G: Actin isoform pattern as a marker of normal or pathological smooth-muscle and fibroblastic tissues. *Differentiation* 33:232–238, 1987
9. GOWN AM, VOGEL AM: Monoclonal antibodies to human intermediate filament proteins II. Distribution of filament proteins in normal human tissues. *Am J Pathol* 114:309–321, 1984
10. SKALLI O, ROPRAZ P, TRZECIAK A, BENZONANA G, GILLESSEN D, GABBIANI G: A monoclonal antibody against α-smooth muscle actin: A new probe for smooth muscle differentiation. *J Cell Biol* 103:2787–2796, 1986
11. TSUKADA T, MCNUTT MA, ROSS R, GOWN AM: HHF35, a muscle actin-specific monoclonal antibody. *Am J Pathol* 127:389–402, 1987
12. OGATA K, KURKI P, CELIS JE, NAKAMURA RM, TAN EM: Monoclonal antibodies to a nuclear protein (PCNA/cyclin) associated with DNA replication. *Exp Cell Res* 168:475–486, 1987
13. BRAVO R, FRANK R, BLUNDELL PA, MACDONALD-BRAVO H: Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. *Nature* 326:515–520, 1987
14. GOWN AM. Anti-actin antibodies: Use in diagnosis, in *Cytoskeletal Proteins in Tumor Diagnosis,* edited by OSBORN M, WEBER K, Cold Spring Harbor Laboratory Press, 1989, p. 79
15. FRANKE WW, MOLL R: Cytoskeletal components of lymphoid organs I. Synthesis of cytokeratins 8 and 18 and desmin in subpopulations of extrafollicular reticulum cells of human lymph nodes, tonsils, and spleen. *Differentiation* 36:145–163, 1987
16. KOCHER O, MADRI JA: Modulation for actin mRNAs in cultured vascular cells by matrix components and TGF-β_1. *In Vitro* 25:424–434, 1989
17. RONNOV-JESSEN L, VAN DEVRS B, CELIS JE, PETERSON OW: Smooth muscle differentiation in cultured human breast gland stromal cells. *Lab Invest* 63:532–543, 1990
18. SCHMITT-GRÄFF A, PAV H, SPAHR R, PIPER HM, SKALLI O, GABBIANI G: Appearance of alpha-smooth muscle actin in human eye lens cells of anterior capsular cataract and in cultured bovine lens-forming cells. *Differentiation* 43:115–122, 1990
19. SAPPINO AP, SCHÜRCH W, GABBIANI G: Biology of disease: Differentiation repertoire of fibroblastic cells: Expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest* 63:144–161, 1990
20. HERMAN IM, D'AMORE PA: Microvascular pericytes contain muscle and nonmuscle actins. *J Cell Biol* 101:43–52, 1985
21. HOLTHÖFER H, MIETTINEN A, PAASIVUO R, LEHTO V-P, LINDER E, ALFTHAN O, VIRTANEN I: Cellular origin and differentiation of renal carcinomas. A fluorescence microscopic study with kidney-specific antibodies, antiintermediate filament antibodies, and lectins. *Lab Invest* 49:317–326, 1983
22. STAMENKOVIC I, SKALLI O, GABBIANI G: Distribution of intermediate filament proteins in normal and diseased human glomeruli. *Am J Pathol* 125:465–475, 1986
23. OOSTERWIJK E, VAN MUIJEN GNP, OOSTERWIJK-WAKKA JC,

WARNAAR SO: Expression of intermediate-sized filaments in developing and adult human kidney and in renal cell carcinoma. *J Histochem Cytochem* 38:385–392, 1990
24. JONES H, STEART PV, DU BOULAY CEH, ROCHE WR: Alpha-smooth muscle actin as a marker for soft tissue tumors: A comparison with desmin. *J Pathol* 162:29–33, 1990
25. RANGDAENG S, TRUONG LD: Comparative immunohistochemical staining for desmin and muscle-specific actin: A study of 576 cases. *Am J Clin Pathol* 96:32–45, 1991
26. FLOEGE J, JOHNSON RJ, GORDON K, IIDA H, PRITZL P, YOSHIMURA A, CAMPBELL C, ALPERS CE, COUSER WG: Increased synthesis of extracellular matrix in mesangial proliferative nephritis. *Kidney Int* 40:477–488, 1991
27. KURKI P, LOTZ M, OGATA K, TAN EM: Proliferating cell nuclear antigen (PCNA)/cyclin in activated human T lymphocytes. *J Immunol* 138:4114–4120, 1987
28. GARCIA RL, COLTRERA MD, GOWN AM: Analysis of proliferative grade using anti PCNA/cyclin monoclonal antibodies in fixed, embedded tissues: Comparison with flow cytometric analysis. *Am J Pathol* 134:733–739, 1989
29. VANDIERENDONCK JH, WIJSMAN JH, KEIJZER R, VAN DE VELDE CJH, CORNELISSE CJ: Cell-cycle-related staining patterns of anti-proliferating cell nuclear antigen monoclonal antibodies: Comparison with BrdUrd labeling and Ki-67 staining. *Am J Pathol* 138: 1165–1172, 1991
30. KAMEL OW, LEBRUN DP, DAVIS RE, BERRY GJ, WARNKE RA: Growth fraction estimation of malignant lymphomas in formalin-fixed paraffin-embedded tissue using anti PCNA/cyclin 19A2. Correlation with Ki-67 labeling. *Am J Pathol* 138:1471–1477, 1991
31. GABBAI FB, WILSON CB, BLANTZ RC: Glomerular hemodynamic consequences of immune injury. *Semin Nephrol* 11:367–372, 1991
32. BORDER WA, OKUDA S, LANGUINO LR, SPORN MB, RUOSLAHTI E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β_1. *Nature* 346:371–374, 1990