Changes in protein profiles during course of experimental glomerulonephritis

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¹Division of Nephrology, Department of Medicine, Medical University of South Carolina, Charleston; ²Department of Medicine, University of Louisville, Louisville, Kentucky; ³Department of Biological Sciences, University of Iowa, Iowa City, Iowa; and ⁴Department of Medicine, Ralph H. Johnson Department of Veterans Affairs Medical Center, Charleston, South Carolina

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Nazeer K, Janech MG, Lin JJ, Ryan KJ, Arthur JM, Budisavljevic MN. Changes in protein profiles during course of experimental glomerulonephritis. Am J Physiol Renal Physiol 296: F186–F193, 2009. First published November 5, 2008; doi:10.1152/ajprenal.90222.2008.—Better characterization of the molecular mechanisms underlying glomerular cell proliferation may improve our understanding of the pathogenesis of glomerulonephritis and yield disease-specific markers. We used two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS) to generate expression profiles of glomerular proteins in the course of anti-Thy-1 nephritis. Glomeruli were isolated from Wistar rats by sieving, and proteins were separated by 2DE. In preliminary studies using normal rats, we identified known glomerular proteins from microfilaments [tropomyosin (Tm)] and intermediate filaments (vimentin and lamin A), proteins involved in assembly (α-actinin-4, F-actin capping protein) and membrane cytoskeletal linking (ezrin), as well as several enzymes (protein disulfide isomerase, ATP synthase, and aldolase). Changes in protein abundance between normal rats and rats in the early phase of anti-Thy-1 nephritis yielded 28 differentially expressed protein spots. MS analysis identified 16 differentially expressed proteins including Tm. Altered Tm abundance in the course of anti-Thy-1 nephritis was confirmed, and specific isoforms were characterized by Western blotting. We demonstrated a complex change in Tm isoform abundance in the course of anti-Thy-1 nephritis. The early mesangio-lytic phase of the disease was characterized by decreased abundance of low-molecular-weight isoforms Tm5a/5b and increased abundance of high-molecular-weight isoforms Tm6, Tm1, Tm2, and Tm3. The late proliferative phase of the disease was associated with increased abundance of isoforms Tm5a/5b, Tm6, and Tm1 and decreased abundance of Tm3. Isoforms Tm4 and Tm5 remained unchanged in the course of this model of experimental glomerulonephritis. Characterization of Tm isoform abundance in the course of clinical glomerulonephritis may identify disease-specific markers.

two-dimensional electrophoresis; mass spectrometry; anti-Thy-1 glomerulonephritis; tropomyosin

GLOMERULONEPHRITIS (GN) remains an important cause of end-stage renal disease (ESRD). Patients with glomerular injury experience significant morbidity and complications. Many forms of GN are associated with proliferation of intrinsic glomerular cells. Although this proliferation could initially be considered a desirable attempt to repair damaged glomeruli, unabated proliferation eventually leads to sclerosis and ultimately glomerular demise. To develop successful treatment strategies for GN, we need a better understanding of the molecular mechanisms underlying glomerular proliferation. Additionally, the elucidation of accompanying changes in the pattern of protein abundance may yield disease-specific markers. Animal models of GN provide a useful tool to understand these mechanisms. The rat model of experimental mesangial proliferative nephritis, anti-Thy-1 nephritis, is characterized by transient but exuberant cellular proliferation and mild matrix expansion. These changes are reminiscent of the mesangial proliferative nephritis seen in humans with IgA nephropathy or focal lupus nephritis. Working with anti-Thy-1 nephritis, Alpers et al. (1) demonstrated that proliferating mesangial cells temporarily undergo phenotypic changes while acquiring the characteristics of myofibroblasts. This is demonstrated by the de novo expression of α-smooth muscle actin. Hugo et al. (7) proposed that mesangial cells need these phenotypic changes in order to repopulate and repair extensively damaged mesangium in the course of GN.

MATERIALS AND METHODS

Unless otherwise specified, all drugs, chemicals, reagents, and antibodies were obtained from Sigma (St. Louis, MO). Animal studies were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina, and all in vivo experi-

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ments were conducted in a humane manner in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Induction of anti-Thy-1 nephritis and glomerulus isolation. Anti-Thy-1 nephritis was induced in 200-g male Wistar rats by a single intravenous injection (1 mg/kg) of the monoclonal antibody OKT-7 (ECACC, Salisbury, UK). An equal number of rats were injected with normal saline and served as a control group of healthy animals without anti-Thy-1 nephritis. For preliminary time course studies of histological changes, animals were killed 24 h and 4, 7, 14, and 28 days after induction of anti-Thy-1 nephritis. For subsequent proeminent studies, rats were killed 7 and 28 days after injection of the anti-Thy-1 antibody. After pentobarbital anesthesia was achieved, animals were perfused with ice-cold PBS through a left ventricular puncture after both renal veins were severed. The lower pole of one kidney from each animal was placed in Carnoy’s solution and processed for histology. With the remaining kidney tissue, glomeruli were isolated by selective sieving as described previously (2). The purity of glomerular histology. With the remaining kidney tissue, glomeruli were isolated both renal veins were severed. The lower pole of one kidney from perfused with ice-cold PBS through a left ventricular puncture after both renal veins were severed. The lower pole of one kidney from each animal was placed in Carnoy’s solution and processed for histology. With the remaining kidney tissue, glomeruli were isolated by selective sieving as described previously (2). The purity of glomerular isolates (>90%) was routinely established by light microscopy. Isolated glomeruli were snap frozen and used for analyses as described below. Animals were kept in metabolic cages for 24 h before death, and urine was collected. Urine protein concentrations were determined with quanTtest red pyrogallol (Quantimetrix, Redondo Beach, CA). Each experimental group comprised at least four rats.

Two-dimensional gel electrophoresis. The isolated glomeruli were ground in a tissue homogenizer in 200 μl of a buffer containing 9 M urea, 4% NP-40, 0.2% Bioyltes, 1% DTG, Benzonase, and the following inhibitors: leupeptin, pepstatin, aprotinin, EDTA, sodium orthovanadate, sodium fluoride, and PMSF. The lysate was then sonicated on ice with three 2-s pulses with a 3-s rest between each pulse. The sonication was repeated every 15 min for 1 h, after which the lysates were centrifuged for 5 min at 750 g and 4°C to remove debris.

Protein concentration was measured with an RC DC protein assay (Bio-Rad, Hercules, CA). Two hundred micrograms of protein was added to a buffer containing 10 mM Tris/HCl, 5 M urea, 2 M thiourea, 2% 3-(3-cholamidopropyl)dimethylammonio)-1-propane-sulfonate (CHAPS), 0.2% Bioyltes, and 50 mM DTT mixed in a 50:50 mixture of trifluoroethanol and ddH2O. The final volume of 200 μl was used to rehydrate an 11-cm IEF strip (pH 4–7, Bio-Rad). Proteins were focused in a Protean IEF cell (Bio-Rad) for 80,000 Vh with a maximum voltage of 8,000 V and a maximum current of 50 μA/strip. After focusing, strips were equilibrated sequentially in buffers containing DTT and iodoacetamide and separated by SDS-polycrlylamide gel electrophoresis on an 8–16% gradient gel with a Criterion Dodeca cell (Bio-Rad). Gels were washed with deionized water, fixed with 10% methanol-7% acetic acid, stained overnight in the dark with Sypro Ruby (Molecular Probes), and imaged on an FX Pro Plus fluorescent imager (Bio-Rad). Images were analyzed with PDQuest software version 7.1.

Protein identification with matrix-assisted laser desorption/ionization time of flight mass spectrometry. Protein spots were picked from the gel with a Proteome Works spot-picking robot (Bio-Rad) and digested with trypsin and a Multiprobe automated digester (Micromass). After sample cleanup with C18 zip tips (Millipore, Bedford, MA), peptides were spotted on a matrix-assisted laser desorption/ionization (MALDI) plate with alpha CN matrix and analyzed on a MALDI-time of flight (TOF) mass spectrometer in reflectron mode. Peptide mass fingerprinting was done against the MSDB database. Mascot Distiller was used for peak picking and Mascot for protein identification. The following assumptions were used: peptide tolerance of 100 ppm, up to 1 missed cleavage, carbamidomethylation of cysteines, variable oxidation of methionines, and rat taxonomy database. Significant identifications were based on MOHSE scores generated by Mascot, where significant identifications were accepted when P < 0.05.

For all identified proteins, subsequent comparison confirmed that their positions on the original 2D gels were in the expected range for isoelectric points and molecular masses.

Protein abundance was determined by analysis of Sypro Ruby staining with PDQuest. To compare differentially expressed proteins, spot and abundance on gels was normalized to the sum of intensities for all valid spots and expressed as parts per million. The abundance of spots between normal animals and animals with anti-Thy-1 nephritis and between animals with anti-Thy-1 nephritis on days 7 and 28 was compared by t-test.

Western blot analysis. Glomerular samples were homogenized in membrane solubilization buffer (4 M urea, 0.1% SDS, 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.1% Triton X-100, and protease inhibitors included in Calbiochem Protein Inhibitor Set III, no. 539134), and 5–20 μg of protein from rats with and without anti-Thy-1 nephritis were loaded on 4–20% gradient one-dimensional SDS-PAGE gels and separated at 200 V. (Some gels were run on 12% gradient gels in MOPS buffer in order to achieve better separation of individual tropomyosin isoforms.) Proteins were transferred onto Immobilon-P membrane (Millipore) with a Criterion Blotter (Bio-Rad) at 100 V for 40 min. After blocking with 5% milk-0.1% Tween 20, the membranes were incubated in blocking buffer overnight at 4°C with anti-Tm antibody. Bands were visualized with horseradish peroxidase-labeled secondary antibody followed by the Pierce enhanced chemiluminescence detection system. Band intensities were measured with a GS 800 densitometer and Quantity One software (Bio-Rad). The quantitative difference in band intensity was assessed by single-factor analysis of variance (ANOVA) followed by Fisher’s least significant difference post hoc analysis. If data were not normally distributed, values were log-transformed before ANOVA analysis. Differences were determined significant at P < 0.05. To ensure equal protein loading, a duplicate gel was run and stained with Coomassie blue, and the areas of each gel lane were compared with Scion Image (Scion). We used the following antibodies: antibody 5441 (Chemicon, Temecula, CA) raised in sheep against the synthetic peptide corresponding to the amino acid sequence of the 9d exon of the Tm a gene (recognizes isoforms 1, 2, 3, 5a, 5b, and 6); monoclonal antibody 311 (Sigma), which recognizes products of exon 1a of the a and β genes and thus isoforms 1, 2, 3, and 6; monoclonal CG3 antibody, which recognizes amino acid sequences 29–44 of exon 1b from the γ gene.

Fig. 1. Histological changes in the course of anti-Thy-1 nephritis [periodic acid Schiff (PAS) stained, ×400 magnification]. A: normal glomerulus. B–D: early-phase glomerular changes: enlarged glomerulus with mesangiolysis (B), microaneurysm (C), and crescent (D). E: late-phase glomerular changes with cellular proliferation and mild matrix expansion.
and thus Tm5 isoform; and antibody LC24, which is specific for isoform 4 (Tm4).

RESULTS

Quantitative differences in glomerular protein abundance in the course of experimental glomerulonephritis. We evaluated the ability of 2DE to detect differences in the abundance of glomerular proteins in the course of anti-Thy-1 GN. First, we determined the evolution of histological changes that occur in this model of GN with time in periodic acid Schiff (PAS)-stained sections of the kidney.

Several days after injection of monoclonal anti-Thy-1 antibody, a complement-mediated mesangiolysis occurred, characterized by extensive depletion of mesangial cells and matrix (Fig. 1B). The glomeruli increased in size, and the most severely affected capillary loops developed microaneurysms (Fig. 1C). Fibrin crescents could also be seen in some glomeruli (Fig. 1D). This “early phase” of anti-Thy-1 nephritis was most pronounced 4–8 days after injection. Over the next several weeks healing of glomerular lesions ensued, with transient but exuberant cellular proliferation and mild matrix expansion. In our hands, this “late phase” of anti-Thy-1 nephritis cellular proliferation was most prominent 4 wk after induction of the disease (Fig. 1E).

We also characterized the extent of proteinuria throughout the course of anti-Thy-1 nephritis. Thy-1 rats displayed significant proteinuria compared with control animals (Fig. 2). Normal male Wistar rats had proteinuria below 20 mg/24 h. Induction of anti-Thy-1 nephritis induced proteinuria in excess of 80 mg/24 h, and this proteinuria had a tendency to be more pronounced in the cellular proliferative phase of the disease.

To discern the molecular basis of the histological changes seen in this model of GN we compared protein abundance in the early phase of the disease to that in healthy control rats. Comparison of spot abundances on 2D gels obtained from control rats and rats at day 7 of anti-Thy-1 nephritis revealed 28 differentially expressed protein spots. Figure 3 shows upregulated and downregulated spots in the diseased animals. We identified 7 of the 28 differentially expressed proteins by MALDI-TOF peptide mass fingerprinting including Tm. A list of the identified differentially expressed proteins is shown in Fig. 4. We focused further investigation on Tm because of the association between its abundance and isoform switching with proliferation and cell motility in other tissues.

Fig. 2. Proteinuria in the course of anti-Thy-1 nephritis. Control Wistar rats without nephritis (filled bars) had proteinuria below 20 mg/24 h. Proteinuria in rats with anti-Thy-1 nephritis (open bars) exceeded 80 mg/24 h throughout the course of the disease.

Fig. 3. Partial images of 2-dimensional (2D) gels obtained from glomerular samples of control rats and rats with anti-Thy-1 nephritis at day 7. A: arrows show a protein spot upregulated in 3 rats with anti-Thy-1 nephritis compared with 3 control rats without nephritis. B: arrows show a protein spot downregulated in 3 rats with anti-Thy-1 nephritis compared with 3 control rats without nephritis.

Differential abundance of tropomyosin isoforms in glomeruli of control rats and rats with anti-Thy-1 nephritis. In the anti-Thy-1 model of GN, proliferating mesangial cells temporarily transform into myofibroblasts, acquiring staining for α-smooth muscle actin. Because of the close structural association of actin with Tm, we decided to explore in more detail the glomerular abundance of Tm in this disease model.
The initial mass spectrometry (MS) analysis of the upregulated Tm spot identified peptides that were most consistent with Tm isoform 6. To confirm the identification and determine the changes in other Tm isoforms, we used Western blotting with four different anti-Tm antibodies. Western blot data were not normalized to housekeeping proteins because proteomic analysis showed differences in several housekeeping proteins; therefore, normalization was maintained by equal sample loading.

Figure 5A depicts results obtained with antibody 5441 that can recognize four high-molecular-weight (HMW) isoforms (1, 2, 3, and 6) and two low-molecular-weight (LMW) isoforms (5a and 5b). We consistently observed that the HMW bands were upregulated in the early phase of anti-Thy-1 nephritis and that their abundances remained increased in the late phase of the disease. Densitometric analysis revealed fourfold increases in their combined abundance both 7 and 28 days after induction of GN compared with controls (Fig. 5B). In this experiment we also observed that the abundance of the LMW isoforms (5a/5b) was depressed to 20% of healthy control abundance in the early phase of nephritis but significantly increased in the late phase of anti-Thy-1 nephritis (Fig. 5C). Several lines of evidence indicate that these LMW varieties were not degradation products. First, their position on the gel was appropriate for their molecular mass. Second, preincubation of anti-Tm antibody with a 100-fold molar excess of peptide to which antibody 5441 was raised completely abolished the band detection, confirming the specificity of the obtained results (data not shown). Finally, we did not detect these LMW isoforms on Western blots obtained with monoclonal antibody 311 specific for only HMW Tm isoforms.

Figure 6 demonstrates data obtained with monoclonal antibody 311, which recognizes isoforms Tm1, Tm2, Tm3, and Tm6. To achieve better separation of individual bands, electrophoresis was performed in MOPS buffer on large-format gels. We identified all four expected bands in glomerular lysates (Fig. 6A). Densitometric analysis demonstrated significant increase in isoforms Tm6 and Tm1 on both day 7 and day 28 after induction of GN compared with healthy control animals (Fig. 6, B and C). In contrast, isoform Tm2 increased early in the course of the disease (day 7) and returned to the level of control on day 28 (Fig. 6D). The abundance of isoform Tm3 showed a significant increase on day 7 and a significant decrease on day 28 of anti-Thy-1 nephritis compared with both control animals without the disease and animals with nephritis in the early phase (Fig. 6E). So in summary, all four HMW isoforms showed an increased abundance in the early mesangio-lytic phase of the disease. However, with the exception of Tm6, the HMW isoforms demonstrated a significantly decreased abundance in the late proliferative phase of the disease compared with the early phase. These experiments demonstrated a complex pattern of Tm isoform abundance in the course of anti-Thy-1 nephritis.

To obtain better insight into the abundance of other LMW Tm isoforms we employed two additional antibodies. Monoclonal antibody CG3 is specific for nonmuscle Tm5. As presented in Fig. 7, we did not find a significant difference in Tm5 abundance in the early or late phase of the disease compared with control rats without the disease. Finally, we did Western blot analysis with antibody LC24, which is specific for Tm4 (Fig. 8). Densitometric analysis revealed no significant change in the abundance of this LMW Tm isoform in the course of anti-Thy-1 nephritis (Fig. 8B).

**DISCUSSION**

We employed a proteomic approach to study protein abundance in the course of the experimental proliferative GN, anti-Thy-1 nephritis. Comparison of glomerular protein abundance on 2DE gels between normal rats and rats with anti-Thy-1 nephritis yielded 28 differentially expressed protein spots. MS analysis identified seven charge forms of four differentially expressed proteins: tropomyosin, H⁺-ATP synthase, α2U-globulin, and 3-mercaptopyruvate sulfotransferase (MST). Subsequent analysis revealed 12 additional differen-
tially expressed proteins in the course of anti-Thy-1 nephritis (Table 1).

In GN, proliferating mesangial cells undergo transient phenotypic transformation into α-smooth muscle actin-positive myofibroblasts (1, 2). Our finding of altered Tm abundance on 2DE gels prompted us to study the abundance of this protein in the course of anti-Thy-1 nephritis in more detail.

The Tm family consists of >40 isoforms with molecular masses ranging between 27 and 40 kDa. The isoforms are products of four genes, αTm, βTm, γTm, and δTm (13). Selected Tm isoforms that are generated by alternate splicing of these genes are summarized in Table 2. Recent evidence suggests that isoforms exert functional differences as well as cellular (tissue) specificity (e.g., muscle isoforms) (5, 18). The repertoire of isoform selection is strictly regulated by as yet unknown mechanisms. Tm associates with actin and constitutes microfilaments, which are primary determinants of cell

![Fig. 5](http://ajprenal.physiology.org/)

Fig. 5. Western blot analysis of glomerular samples from control rats and rats with anti-Thy-1 nephritis at day 7 (early phase) and day 28 (late phase) probed with antibody 5441. This antibody recognizes 4 high-molecular-weight (HMW) tropomyosin isoforms (Tm1, Tm2, Tm3, and Tm6) and 2 low-molecular-weight (LMW) isoforms (Tm5a/5b). A: representative blot demonstrates that HMW Tm isoforms are upregulated in both early and late phase of anti-Thy-1 nephritis compared with control rats. In contrast, LMW isoforms are upregulated only in the late phase of the disease. B: relative abundance of HMW Tm isoforms. Densitometric analysis revealed 394% and 391% increase in expression of HMW tropomyosin isoforms in the early and late phases of anti-Thy-1 nephritis, respectively, compared with control rats without nephritis (C) (ANOVA P < 0.05; Fisher’s least significant difference *P < 0.05). C: relative abundance of LMW Tm isoforms. Densitometric analysis revealed 500% decrease in isoforms Tm5a/5b in the early phase of anti-Thy-1 nephritis compared with control rats. A significant 555% increase in their abundance compared with control rats was noted in the late phase of the disease (ANOVA *P < 0.05; #P < 0.01 vs. control).

![Fig. 6](http://ajprenal.physiology.org/)

Fig. 6. Western blot analysis of glomerular samples from control rats and rats with anti-Thy-1 nephritis at day 7 and day 28 probed with monoclonal antibody 311. This antibody recognizes 4 HMW Tm isoforms (Tm1, Tm2, Tm3, and Tm6). A: Western blot demonstrating 4 bands (arrows indicate individual bands). B: densitometric analysis of Tm6 isoform revealed 585% and 440% increase in abundance in early (day 7) and late (day 28) phases of anti-Thy-1 nephritis, respectively, compared with control (ANOVA *P < 0.01 vs. control). C: densitometric analysis of Tm1 band revealed an almost 10-fold increase in abundance in the early phase (day 7) and 375% increase in abundance in the late phase (day 28) of anti-Thy-1 nephritis compared with control rats without disease (ANOVA *P < 0.01 vs. control). D: a significant 350% increase in the abundance of Tm2 isoform was noted only in the early phase (day 7). The abundance returned to baseline in the late phase (day 28) (ANOVA *P < 0.01 vs. control). E: a 2-fold increase in the abundance of Tm3 isoform was observed in the early phase (day 7). In the late phase (day 28), the abundance of this isoform decreased significantly below that seen in control rats without nephritis (ANOVA *P < 0.01 vs. control).

**Table 1.**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression Pattern</th>
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<tbody>
<tr>
<td>Tm1, Tm2, Tm3</td>
<td>Upregulated in early and late phases</td>
</tr>
<tr>
<td>Tm5a/5b</td>
<td>Downregulated in early phase</td>
</tr>
<tr>
<td>Tm6</td>
<td>Upregulated in late phase</td>
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**Table 2.**

<table>
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<tr>
<th>Tm Isoform</th>
<th>Description</th>
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<tbody>
<tr>
<td>Tm1</td>
<td>40 kDa high-molecular-weight isoform</td>
</tr>
<tr>
<td>Tm2</td>
<td>35 kDa high-molecular-weight isoform</td>
</tr>
<tr>
<td>Tm3</td>
<td>30 kDa high-molecular-weight isoform</td>
</tr>
<tr>
<td>Tm4</td>
<td>27 kDa low-molecular-weight isoform</td>
</tr>
<tr>
<td>Tm5a/5b</td>
<td>25 kDa low-molecular-weight isoform</td>
</tr>
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**Figures:**

- **Fig. 5** shows Western blot analysis of glomerular samples from control rats and rats with anti-Thy-1 nephritis at day 7 (early phase) and day 28 (late phase) probed with antibody 5441. This antibody recognizes 4 high-molecular-weight (HMW) tropomyosin isoforms (Tm1, Tm2, Tm3, and Tm6) and 2 low-molecular-weight (LMW) isoforms (Tm5a/5b). A: representative blot demonstrates that HMW Tm isoforms are upregulated in both early and late phase of anti-Thy-1 nephritis compared with control rats. In contrast, LMW isoforms are upregulated only in the late phase of the disease. B: relative abundance of HMW Tm isoforms. Densitometric analysis revealed 394% and 391% increase in expression of HMW tropomyosin isoforms in the early and late phases of anti-Thy-1 nephritis, respectively, compared with control rats without nephritis (C) (ANOVA P < 0.05; Fisher’s least significant difference *P < 0.05). C: relative abundance of LMW Tm isoforms. Densitometric analysis revealed 500% decrease in isoforms Tm5a/5b in the early phase of anti-Thy-1 nephritis compared with control rats. A significant 555% increase in their abundance compared with control rats was noted in the late phase of the disease (ANOVA *P < 0.05).

- **Fig. 6** shows Western blot analysis of glomerular samples from control rats and rats with anti-Thy-1 nephritis at day 7 and day 28 probed with monoclonal antibody 311. This antibody recognizes 4 HMW Tm isoforms (Tm1, Tm2, Tm3, and Tm6). A: Western blot demonstrating 4 bands (arrows indicate individual bands). B: densitometric analysis of Tm6 isoform revealed 585% and 440% increase in abundance in early (day 7) and late (day 28) phases of anti-Thy-1 nephritis, respectively, compared with control (ANOVA *P < 0.01 vs. control). C: densitometric analysis of Tm1 band revealed an almost 10-fold increase in abundance in the early phase (day 7) and 375% increase in abundance in the late phase (day 28) of anti-Thy-1 nephritis compared with control rats without disease (ANOVA *P < 0.01 vs. control). D: a significant 350% increase in the abundance of Tm2 isoform was noted only in the early phase (day 7). The abundance returned to baseline in the late phase (day 28) (ANOVA *P < 0.01 vs. control). E: a 2-fold increase in the abundance of Tm3 isoform was observed in the early phase (day 7). In the late phase (day 28), the abundance of this isoform decreased significantly below that seen in control rats without nephritis (ANOVA *P < 0.01 vs. control).
It is now widely accepted that nonmuscle Tm protects actin filaments from the depolymerizing action of DNase I and actin depolymerizing factor (ADF)/cofilin and the severing action of gelsolin (13, 18).

No information about Tm isoform behavior in the course of GN is currently available. We have found a distinct pattern of Tm isoform abundance in the course of anti-Thy-1 nephritis (Fig. 9). In the early phase of the disease (day 7), characterized by a depletion of mesangial cells and matrix, we found decreased abundance of the LMW isoforms Tm5a/5b and increased abundance of four HMW isoforms (Tm6, Tm1, Tm2, Tm3). At the peak of the cellular proliferative phase (day 28), the abundance of isoforms Tm6 and Tm1 remained increased while the abundance of isoform Tm2 returned to baseline. In this phase of the disease LMW isoforms Tm5a/5b are elevated and HMW Tm3 depressed compared with control glomeruli without nephritis. We found no change in the abundance of LMW isoforms Tm5 and Tm 4 in either phases of anti-Thy-1 nephritis. So it appears that in the course of anti-Thy-1 nephritis, only products of Tm genes undergo changes in abundance, possibly indicative of altered expression.

The pathophysiological significance of the observed Tm isoform change is not clear. We can only speculate on the basis of changes in expression reported in other cells and pathological processes. LMW isoform 5, and not HMW isoforms 1, 2, and 3, is found on the leading edge of fibroblasts, suggesting a role in membrane organization and motility (12). Therefore, the increased abundance of LMW isoforms Tm5a/5b during the proliferative phase of the nephritis would not have been unexpected. In anti-Thy-1 nephritis, phenotypic transformation of cells for migration, proliferation, and cell-cell interactions is essential in the process of restoring damaged mesangium. PDGF was shown to play a major role in these processes (9).

In addition to the potential role in cytokinesis, the 5a/5b isoforms that were elevated in our study are involved in trafficking of transport proteins to the plasma membrane (3, 4). Such properties may be important in the cellular response to injury. We cannot rule out that the changes in Tm 5a/5b were the result of initial depletion and subsequent proliferation of mesangial cells. However, the dichotomy of change in HMW Tm isoforms and the absence of change in two other LMW isoforms (Tm4 and Tm5) argue that such a possibility cannot be the sole explanation for the findings we observed.

The behavior of HMW Tm isoforms in the course of anti-Thy-1 nephritis appears to be more complicated. Although morphology. It is now widely accepted that nonmuscle Tm protects actin filaments from the depolymerizing action of DNase I and actin depolymerizing factor (ADF)/cofilin and the severing action of gelsolin (13, 18).

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transformed and malignant cells require downregulation of HMW Tm isoforms for invasion and metastasis (reviewed in Ref. 16), exposure of smooth muscle cells to PDGF led to an increase in levels of Tm3 in lipid rafts, resulting in proliferation and migration (14). Similarly PDGF-mediated spreading of glioma cells required relatively high Tm1 abundance and elevated Tm2 and Tm3 RNA levels (11). Of particular interest are our findings of significant decrease in abundance of HMW Tm1, Tm2, and Tm3 isoforms in the late cellular proliferative phase compared with the early mesangiolytic phase. It appears that glomerular cells share some characteristics of transformed/malignant cells and some of smooth muscle cells.

At this point we do not know which intrinsic glomerular cell(s) is/are responsible for altered tropomyosin abundance in the course of anti-Thy-1 nephritis. The early phase of the disease is associated with the exudation of macrophages; however, it is difficult to discern whether these cells would affect Tm profiles in glomeruli. First, macrophages express only LMW Tm isoforms (6, 15). Second, they are present only in the early phase of anti-Thy-1 nephritis, when LMW Tm isoforms are either depressed or unchanged.

To discern in more detail the pathophysiological role of Tm isoforms and develop new mechanistic theories of their involvement in the course of glomerular injury will require experiments with multiple time points and fine characterization of the cells involved. Our goal, however, was to describe the molecular phenotype underlying the most pronounced and unique morphological changes in this model of GN and propose potential clinical markers. Because depletion of mesangial cells and matrix precedes the cellular proliferation phase and the latter predicts recovery, detailed characterization of expressed proteins may be not only a reflection of underlying changes but also a predictor of future behavior. In that respect, precise mapping of sequential changes in the expression of Tm1, Tm2, and Tm3 isoforms from mesangiolytic to proliferative phase may prove important.

Regardless of the pathophysiological significance of Tm isoform change in the course of GN, the distinct pattern of their abundance may prove useful as a clinical marker of glomerular injury. It would be reasonable to extend the study of Tm isoform abundance to kidney biopsies and urine from patients with GN and correlate findings with response to treatment and outcome.

In conclusion, the present study is the first attempt to examine global changes in protein abundance in the course of experimental GN. Our data show the feasibility of 2DE and MS in the development of protein abundance profiles of glomerular injury and the potential for better understanding of pathogenesis of GN. Recently, two studies employed a cDNA microarray approach in search of gene expression profiles in the course of anti-Thy-1 nephritis (17, 19). Both Sadlier et al. (17) and Tsuji et al. (19) found the largest number of differentially expressed genes in the mesangiolytic phase and the peak mesangial cell proliferation phase of the disease, respectively, supporting our choice to search for potential markers at these points in the course of anti-Thy-1 nephritis. Sadlier et al. (17) found a large number of genes involved in development, extracellular matrix, metabolism, transcription, and signal transduction that were perturbed during disease. It is interesting that among 80 genes that demonstrated the most striking up- and downregulation in the course of anti-Thy-1 nephritis only one protein, actin, is shared with our identification by a proteomic approach. They were able to identify many transcription factors and cell signaling molecules whose abundance is too low to be detected by the methodology we employed. At present, it is clear that genomic and proteomic approaches are complementary and that both may be required to obtain the whole mosaic of protein expression in the course of glomerular injury. With the development of more sensitive and easily quantifiable proteomic techniques, a direct protein approach can offer several advantages that include detecting not only differences in protein abundance but also site-specific changes in posttranslational modifications. The finding of clinically useful protein markers makes such an approach desirable as well. Translation of information obtained in animal models with both genomic and proteomic techniques for characterization of the pattern of protein expression to the course of human forms of GN may yield disease-specific markers.

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