Glomerular Expression of Dystroglycans Is Reduced in Minimal Change Nephrosis But Not in Focal Segmental Glomerulosclerosis

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Abstract. Extensive flattening of podocyte foot processes and increased permeability of the glomerular capillary filter are the major pathologic features of minimal change nephrosis (MCN) and focal segmental glomerulosclerosis (FSGS). Adhesion proteins anchor and stabilize podocytes on the glomerular basement membrane (GBM), and presumably are involved in the pathogenesis of foot process flattening. Thus far, α3 β1-integrin was localized to basal cell membrane domains. In this report, α- and β-dystroglycan (DG) were detected at precisely the same location by immunoelectron microscopy, and the presence of α- and β-DG chains was confirmed by immunoblotting on isolated human glomeruli. Because the major DG binding partners in the GBM (laminin, agrin, perlecan), and the intracellular dystrophin analogue utrophin are also present in podocytes, it is therefore plausible that podocytes use precisely actin-guided DG complexes at their “soles” to actively govern the topography of GBM matrix proteins. Expression of the α/β-DG complex was reported to be reduced in muscular dystrophies, and therefore a search for similar pathologic alterations in archival kidney biopsies from patients with MCN (n = 16) and FSGS (n = 8) was conducted by quantitative immunoelectron microscopy. The density of α-DG on the podocyte’s soles was significantly reduced to 25% in MCN, whereas it was not different in normal kidneys and FSGS. The expression of β-DG was reduced to >50% in MCN, and was slightly increased in FSGS. Levels of DG expression returned to normal in MCN after steroid treatment (n = 4). Expression of β1-integrin remained at normal levels in all conditions. These findings point to different potentially pathogenic mechanisms of foot process flattening in MCN and FSGS.

Adhesion of podocytes to the glomerular basement membrane (GBM) is critically associated with the correct filter characteristics of the glomerular capillary wall, as supported by the finding that proteinuria is invariably associated with flattening of foot processes and occasionally also detachment of podocytes (1,2). The primary glomerular filter was localized to the GBM (3), and presumably is maintained by fibrillar and charged GBM matrix proteins arranged in a complex three-dimensional pattern. It is not known, however, whether correct alignment of matrix components is achieved by self assembly (4), or actively regulated by podocytes. Because the basal cell membrane domain of foot processes is directly attached to the GBM, it is of some interest to map and understand molecular interactions between matrix proteins and appropriate podocyte cell membrane receptors. Thus far, only two types of adhesion proteins have been localized unambiguously by immunoelectron microscopy, i.e., α3 β1-integrin (5,6) and a transmembrane heparan sulfate proteoglycan of the syndecan family (7), and there is indirect evidence that α3 β1-integrin is involved in the stabilization of foot processes (8,9).

In this study, we define the glomerular localization of yet another class of adhesion proteins, the α- and β-dystroglycans (DG), which form transmembrane complexes in muscle and several nonmuscle cells (10,11). DG was previously observed in a GBM pattern by immunofluorescence (12,11). Here we have precisely localized α- and β-DG subunits by immunoelectron microscopy to the basal cell membrane domain of foot processes. In addition, we provide evidence that podocytes contain utrophin, a major intracellular binding partner of β-DG, by indirect immunofluorescence. To detect potential alterations of DG expression under pathologic conditions, we have examined its distribution in archival human kidney biopsies of minimal change nephrosis (MCN) before and after steroid treatment, and of segmental focal glomerular sclerosis (FSGS), and we have compared it with α3 β1-integrin. MCN and FSGS were chosen because the primary defect in these...
diseases is thought to reside in podocytes, and causes extensive flattening of foot processes and nephrotic-range proteinuria.

**Materials and Methods**

**Patients and Renal Biopsies**

Indirect immunohistochemistry was performed on paraffin sections of archival renal biopsies of 16 cases of clinically and morphologically typical MCN (five children, ages 2 to 11, and 11 adults, ages 24 to 67), and eight cases of FSGS (Table 1) that were clinically classified as idiopathic. All patients selected showed nephrotic-range proteinuria, and normal or only slightly elevated levels of serum creatinine (Table 1). Routine electron microscopy was used in all cases to monitor foot process morphology. MCN was diagnosed by the standard criteria (13) of extensive foot process flattening, in the absence of other pathologic glomerular changes, and nephrotic-range proteinuria without impairment of renal function. Four cases of MCN biopsies taken before and after successful steroid treatment were examined (Table 2). In biopsies from eight patients, FSGS was defined by typical segmental glomerular obsolescence, adhesion of the glomerular tuft to Bowman’s capsule, extensive foot process flattening by electron microscopy, and nephrotic-range proteinuria. As controls, four samples with normal glomerular morphology from tumor nephrectomies were used.

**Antibodies**

Mouse monoclonal IgG specific for the C-terminal 16 amino acids of human β-DG, anti-utrophin antibody (raised against a recombinant N-terminal 261 amino acid peptide), and monoclonal anti-human dystrophin IgG were obtained from Novocastra Laboratories (Newcastle, United Kingdom). Monoclonal IgG against rabbit α-DG was from Upstate Biotechnology (Lake Placid, NY), and against human β1-integrin from Transduction Laboratories (Lexington, KY), and provided by Dr. Robert Pytela (Lung Research Center, UCSF, San Francisco, CA). Affinity-purified rabbit anti-mouse IgG was from Dako (Copenhagen, Denmark), and sheep anti-rabbit IgG 10 nm immunogold conjugate was from Amersham (Amersham, United Kingdom).

### Table 1. Visual estimate of the labeling intensity of glomeruli immunostained for α- and β-dystroglycan, and β1-integrina

<table>
<thead>
<tr>
<th>Category and Patient (gender, age, yr)</th>
<th>FP Flattening (%)</th>
<th>Proteinuria/Creatinine</th>
<th>Glomerular α-DG</th>
<th>β-DG</th>
<th>β1-Integrin</th>
<th>Tubular α-DG</th>
<th>β-DG</th>
<th>β1-Integrin</th>
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<tbody>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EV (M, 36)</td>
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<td>0/0.4</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<td>++</td>
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<tr>
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<td>0/ND</td>
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<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
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<td>+++</td>
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<td>+++</td>
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<tr>
<td>HS (F, 52)</td>
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<td>0/ND</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>HF (M, 67)</td>
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<td>8/1.9</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
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<tr>
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<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
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<tr>
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<td>+/−</td>
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<td>+/−</td>
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<tr>
<td>FG (F, 30)</td>
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<td>4.9/1.0</td>
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<td>++</td>
<td>ND</td>
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<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
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<td>BB (F, 27)</td>
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<td>8/1.3</td>
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<td>+/−</td>
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<td>SH (M, 61)</td>
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<td>5.5/1.5</td>
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<td>+/−</td>
<td>+/−</td>
<td>ND</td>
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<td>+++</td>
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<tr>
<td>BJ (M, 56)</td>
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<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<td>DF (M, 7)</td>
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<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<td>FSGS</td>
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<td></td>
</tr>
<tr>
<td>FG (M, 43)</td>
<td>90</td>
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<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>PJ (M, 33)</td>
<td>100</td>
<td>3.5/0.6</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PA (M, 43)</td>
<td>80</td>
<td>5/3.5</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<td>AJ (F, 60)</td>
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<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>DV (F, 42)</td>
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<td>5/0.9</td>
<td>ND</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>FI (F, 64)</td>
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<td>13/0.9</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>TJ (M, 65)</td>
<td>90</td>
<td>3/1.6</td>
<td>ND</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>SM (M, 39)</td>
<td>100</td>
<td>13/1</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>ND</td>
<td>+++</td>
<td>+++</td>
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</tbody>
</table>

* Kidney biopsies from 16 patients with MCN (five children, 11 adults), eight patients with FSGS, and four normal kidneys were evaluated independently by two examiners. +++ indicates the highest labeling intensity observed in normal controls (compare with Figure 1, A through C). Four additional MCN patients are listed in Table 2. The degree of foot process flattening was estimated from routine electron micrographs of at least three glomeruli per case. ND indicates “not determined,” usually because of shortage of biopsy material. Proteinuria is expressed as g urinary protein/24 h, and serum creatinine as mg/100 ml.

FP, foot process; DG, dystroglycan; MCN, minimal change nephrosis; FSGS, focal segmental glomerulosclerosis.
Immunoblotting

Glomeruli were isolated from renal cancer nephrectomy specimens by graded sieving, lysed in sodium dodecyl sulfate sample buffer, electrophoresed on 10% gels, and transferred onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Human skeletal muscle was removed from laryngectomy specimens and was directly dissolved in sodium dodecyl sulfate sample buffer. Immunoblotting was performed with anti-α- or β-DG antibodies (5 to 10 μg IgG/ml), and detection was carried out with an enhanced chemiluminescence kit (Bio-Rad, Richmond, CA).

Immunohistochemistry

Two-micrometer-thick paraffin sections of archival kidney biopsies were dewaxed, rehydrated, microwaved (600 W, 10 min), and processed for indirect immunohistochemistry, as described (14). As secondary reagent, a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) was used. In control experiments, incubation with the primary antibodies was omitted, or they were replaced by irrelevant IgG of the same subclasses. Urophin was localized in unfixed cryostat sections of normal human kidney by indirect immunofluorescence.

Labeling intensity of α- and β-DG- and β1-integrin-labeled sections of kidney biopsies was estimated by two independent reviewers, using normal controls and the labeling intensity of tubular basement membranes in the same sections as relative standards. At least six glomeruli per biopsy were evaluated at low-power magnification to assess the labeling intensity of whole glomeruli only and disregarding focal intraglomerular variations. The reviewer’s scores were averaged and expressed from +++ to 0.

Immunoelectron Microscopy

Immunogold electron microscopy was performed on ultrathin frozen sections of kidney biopsies from patients with MCN and FSGS, as well as from healthy control subjects. Briefly, pieces of biopsies were fixed in 4% freshly prepared formaldehyde (by depolymerization of paraformaldehyde), 0.1% distilled glutaraldehyde (Merck, Darmstadt, Germany) in 100 mM phosphate buffer, pH 7.2, for 6 to 12 h at 4°C, soaked in sucrose, and frozen and stored in liquid nitrogen. Ultrathin frozen sections were processed for indirect immunogold labeling, as described (15). Alternatively, biopsies were embedded in Lowicryl K4M resin, and ultrathin sections were labeled by an indirect immunogold protocol (16). In control experiments, incubation with primary antibodies was omitted, or they were replaced by irrelevant IgG of the same subclasses.

Quantitative Immunelectron Microscopy

The number of gold particles per micrometer of basal cell membrane was determined on electron micrographs of Lowicryl K4M sections that permit evaluation of relatively large glomerular segments (15). Cell membranes from at least two glomeruli of three different patients in each group were evaluated. The results for α- and β-DG were based on the evaluation of the following total membrane lengths: 2046 and 3784 μm in control subjects, 1643 and 3770 μm in MCN, and 1651 μm in FSGS. For β1-integrin, the lengths were: 2553 μm in control subjects, 2553 μm in MCN, and 1651 μm in FSGS.

Statistical Analyses

The mean values were calculated, and SD and significances were determined by unpaired t test. A P value <0.05 was regarded as statistically significant.

Results

Dystroglycans and β1-Integrin Localization in Normal Human Kidneys

In normal kidney, α- and β-DG are coexpressed in glomerular peripheral capillary loops in a GBM-like pattern (Figure 1, A and B), as reported previously (11,12). Proximal tubules and vascular smooth muscle cells also expressed both DG. Immunostaining with anti-β1-integrin IgG resulted in a similar labeling pattern, with the addition of mesangial reactivity (Figure 1 C). Interstitial blood vessels were stained similar to DG. In

Table 2. Estimate of the labeling intensity of glomeruli immunostained for α-dystroglycan of patients with minimal change nephrosis, before and after clinically successful steroid therapy

<table>
<thead>
<tr>
<th>Patient (gender, age, yr)</th>
<th>Steroids</th>
<th>Proteinuria/Creatinine</th>
<th>FP Flattening (%)</th>
<th>Glomerular α-DG</th>
<th>Tubular α-DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB (F 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biopsy A</td>
<td>X</td>
<td>7/0.5</td>
<td>100</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>biopsy B</td>
<td>X</td>
<td>0.2/0.6</td>
<td>0</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>GB (M 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biopsy A</td>
<td>X</td>
<td>7.8/0.7</td>
<td>100</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>biopsy B</td>
<td>X</td>
<td>0.2/0.8</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>SW (M 38)</td>
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<td>biopsy A</td>
<td>X</td>
<td>3.6/0.9</td>
<td>100</td>
<td>+</td>
<td>++</td>
</tr>
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<td>biopsy B</td>
<td>X</td>
<td>0.1/0.9</td>
<td>0</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>AK (F 11)</td>
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<td></td>
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<tr>
<td>biopsy A</td>
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<td>6.5/1.2</td>
<td>100</td>
<td>++</td>
<td>++</td>
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<td>biopsy B</td>
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<td>0.3/0.9</td>
<td>0</td>
<td>+++</td>
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</tbody>
</table>

*The degree of foot process flattening was estimated from routine electron micrographs of at least three glomeruli per case. ND indicates “not determined,” usually because of shortage of biopsy material. Proteinuria is expressed as g urinary protein/24 h, and serum creatinine as mg/100 ml. Abbreviations as in Table 1.
control experiments, the anti-DG antibodies outlined the basal membranes of skeletal muscle fibers (data not shown).

**Utrophin Localization in Glomeruli**

In a preliminary investigation, we have tried to localize known intracellular binding partners of β-DG in glomeruli in unfixed cryostat sections of normal kidney by immunofluorescence. Utrophin was localized in a coarse granular pattern, outlining the contours of peripheral capillary loops (Figure 2), at least suggesting that utrophin is present within the glomerulus. Dystrophin was localized previously within the mesangial region (17), but not in peripheral capillary loops.

**α- and β-DG Expression in Glomeruli**

Immunoblotting with peptide-specific α- and β-DG antibodies on lysates of isolated human glomeruli and human skeletal muscle resulted in immunolabeling in both tissues of a 156-kD protein with the antibody specific for α-DG, and of 43 kD for β-DG (Figure 3).
**α- and β-DG Localization at the Base of Foot Processes**

Immunoelectron microscopy of normal human glomeruli on ultrathin cryosections or K4M Lowicryl-embedded material revealed specific, uniformly dense labeling exclusively on the podocyte’s and foot processes’ basal cell membrane that attaches to the GBM (Figures 4 and 6). The localization of α- and β-DG were identical, however, immunogold labeling intensity obtained with antibodies to α-DG were consistently stronger than with β-DG in K4M sections (Figures 5 and 7). No labeling was obtained on the cell membranes of endothelial cells, and few gold particles were localized to mesangial cell membranes. β1-Integrin was found on the podocyte’s basal membrane domains, as well as on endothelial cells, as described (5).

**Firm α-DG Adherence to the GBM**

Occasionally, the podocytes were mechanically removed in ultrathin cryostat sections, leaving behind an intact GBM and endothelium. In these regions, dense specific immunogold labeling was observed for α-DG (Figure 4B), while gold particles indicating β-DG were detected only sporadically (data not shown).

**Reduced Expression of Glomerular DG in MCN But Not in FSGS**

When paraffin sections of renal biopsies with normal glomeruli (n = 4) and from patients with MCN (n = 16) were

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**Figure 2.** Utrophin is localized in cryostat sections of normal kidney by indirect immunofluorescence in glomerular capillary loops (CL) in a linear pattern (arrowheads) similar to that found for dystroglycans (12). Magnification, ×2400.

**Figure 3.** Detection of α-dystroglycan (lanes A and C) and β-dystroglycan (lanes B and D) in isolated human glomeruli (lanes A and B) and in human skeletal muscle (lanes C and D). In both tissues, the antibodies used subsequently for immunocytochemistry specifically recognize α-dystroglycan with apparent molecular weight of 156 kD (lanes A and C), and β-dystroglycan, respectively, with molecular weight of 43 kD (lanes B and D).

**Figure 4.** Localization of α-dystroglycan in normal human kidney by immunogold labeling on ultrathin frozen sections. α-Dystroglycan is distributed in high density exclusively on the base of the foot processes (arrowheads in A) in an evenly dispersed pattern that is particularly well-recognized in grazing sections (D). Endothelial cells are not labeled. (B) In this ultrathin frozen section, the podocyte was mechanically removed, leaving intact the GBM and the endothelium (E). There is dense specific labeling for α-dystroglycan in the lamina rara externa, indicating that α-dystroglycan is firmly bound there and ripped out of the podocyte cell membrane by the cutting process. (C) Basal portion of a proximal tubule showing specific labeling on the basal cell membrane (arrowheads) toward the basement membrane (BM). Magnification, ×45,000.
immunostained in parallel with anti-α- or β-DG antibodies, the majority of MCN glomeruli showed a much lower signal than normal glomeruli (Figure 1, G and H, Table 1). This difference became particularly apparent when the labeling density of glomeruli was compared to tubular basement membranes within the same section. By contrast, no gross difference in glomerular labeling intensity was observed in sections of 14 cases of FSGS (Figure 1, D and E).

To judge more objectively the expression of the DG than by light microscopic immunohistochemistry, we used quantitative immunogold electron microscopy (15). Renal biopsies of three normal kidneys and three cases of MCN and FSGS each were embedded in K4M Lowicryl (Figures 7 and 8). K4M sections were used for quantification of gold particles because they permitted the evaluation of large, continuous, and uniformly immunolabeled segments of glomeruli. Ultrathin sections were processed in parallel for immunogold surface labeling to minimize technical inconsistencies. The major result was that in MGN the density of gold particles at the base of the foot processes was significantly reduced to 25% for α-DG and

Figure 5. Localization of α-dystroglycan in normal human kidney by immunogold labeling on Lowicryl-K4M sections. In this grazing section, the high density of α-dystroglycan on the basal membrane domains of podocytes is apparent. US, urinary space; E, endothelium; P, podocyte. Magnification, ×25,000.

Figure 6. Localization of β-dystroglycan in normal human glomeruli in ultrathin frozen sections. β-Dystroglycan is specifically localized in the cell membrane domain at the base of the foot processes (arrowheads), but not on the endothelium (E). Magnification, ×45,000.
>50% for β-DG when compared with controls (100%). By contrast, no difference in labeling intensity was observed in FSGS (n = 8) (Figure 9, Table 2), however, clustering of DG in basal podocyte membranes was observed, especially with anti-α-DG antibodies that labeled K4M sections more intensely than antibodies to β-DG (Figure 7B). The density of β₁-integrin was identical in normal glomeruli and in MCN and FSGS (Figure 1, F and I).

**Restoration of Glomerular DG in MCN by Steroid Treatment**

Biopsies of four patients (three children, one adult) with MCN before and after clinically successful steroid therapy were immunolabeled with α-DG antibodies to indicate the DG complex. This was warranted because α- and β-DG were always expressed concomitantly (Figure 1). In all cases, the relative intensity of the glomerular histochemical reaction was very low before steroid treatment, and returned to normal levels after therapy, along with the restoration of normal foot processes (Table 2).

**Discussion**

MCN and FSGS are considered “podocyte diseases,” mainly because their principal pathologic feature is extensive flattening of foot processes, accompanied by nephrotic-range proteinuria (1,2,13). Both diseases may be related to each other (18), and they presumably represent a common pathway of different pathologic injuries to podocytes. FSGS is clinically characterized by progression and steroid resistance, whereas MCN is steroid-sensitive and disappears in most cases with adolescence. The severe derangement of podocyte foot processes raised the possibility that the interaction of extracellular matrix proteins via transmembrane connectors to the cytoskeleton could be defective, and thus mapping of podocyte adhesion proteins is of potential interest. The current inventory comprises α₃β₁-integrin (5,6) and a heparan sulfate proteoglycan of the syndecan family (7). Here we have localized α- and β-DG to foot processes, and provided evidence for its faulty expression in MGN that is reversed by steroid treatment.

Among the established transmembrane linkers of the cytoskeleton to extracellular matrix, the DG complex is particularly interesting, because its sarcolemmal expression is dramatically reduced in several forms of muscular dystrophies (10,11). DG consists of two polypeptide chains, designated α- and β-DG, that are synthesized as single precursor and posttranslationally processed and cleaved to produce the transmembrane 43-kD β and the extracellular 156-kD α subunits. The α
subunit is noncovalently bound to the ectodomain of the β subunit, and carries several O-linked, sialomucin-like carbohydrate side chains (19) that provide neuraminidase-sensitive binding sites for laminin G subunits that are also present in the GBM proteoglycans agrin and perlecan (20–22). The main intracellular binding partners of β-DG are dystrophin and its homologue utrophin that directly interact with actin (23). Although dystrophin is restricted to myocytes and in kidney to collecting tubules and the glomerular mesangium (17), utrophin and short forms of dystrophin are widely expressed in nonmuscle cells, including podocytes (Figure 2) (24,25). Thus, podocytes are endowed with a set of components of a transmembrane DG complex that includes utrophin, α- and β-DG, and laminin and proteoglycans in the GBM.

Besides providing cellular anchorage to the extracellular matrix, DG complexes profoundly influence the organization of basement membranes by orchestrating the positions of matrix proteins (26). For example, genetic “knockout” of DG results in a lethal phenotype that is caused by insufficient organization of Reichert’s membrane (27). Reduced surface expression of the DG complex on cultured muscle cells by RNA antisense constructs causes failure of myocytes to organize an orderly basement membrane (26). Presumably for the same reason, addition to culture media of a monoclonal, ligand-blocking anti-α-DG antibody inhibited formation of glomeruli in an in vitro renal organogenesis system (28). Spatial positioning of DG within muscle cell membranes may be influenced by the cytoskeleton (29). Thus, the final organization of mature basement membranes is presumably a result of both spontaneous matrix protein assembly (4) and precisely controlled, nonrandom distribution by actin-guided DG complexes on cell membranes (26,29). Taken together with the findings that DG is exclusively localized to the basal cell membrane domains of podocytes and firmly adheres to the lamina rara externa of the GBM, the intriguing possibility exists that podocyte-controlled, actin-mediated, nonrandom positioning of DG complexes actively controls the arrangement of GBM matrix proteins, and thus influences the permeability of this prime glomerular filter barrier. The “division of labor” of podocyte adhesion between the DG complex and β1-integrin is not clear.

A major finding of this study was the precisely targeted localization of α- and β-DG to the basal cell membrane of foot processes. This “sole” of foot processes is a highly specialized domain that contains, besides integrin and DG, the membrane mucoprotein podoplanin (15), and on its edges the slit diaphragm-associated proteins p51 (30) and nephrin (31), all of which were implicated in triggering foot process flattening.
The DG complex is known to support the formation of large, two-dimensional membrane protein complexes such as ion channels that are recruited by the PDZ domains of β-DG-linked utrophin (33). The basal cell membrane of foot processes is exquisitely rich in cholesterol (34), and it is possible that it contains "rafts" of membrane proteins (35) with reduced lateral membrane mobility and physical and functional coupling. DG and utrophin would be good candidates for the organization of rafts in a podocyte-governed, cytoskeleton-dependent manner, similar to their role in the assembly of neuromuscular synapses (36).

Is the expression of the podocyte’s DG complex changed in glomerular diseases? Experimental animal models for flattening of foot processes are complex and may involve pathogenic mechanisms different from human diseases. Therefore, we have examined here the expression and distribution of DG directly in human renal biopsy specimens with MCN and FSGS. We have focused on these diseases because podocyte flattening appears to be the primary cause of the functional glomerular abnormalities. An obvious disadvantage was the restriction to archival biopsy material that permits immunohistochemical analysis only, because not enough glomerular material for RNA extraction was recovered by microdissection. With this limitation, we have used here carefully calibrated light microscopic immunocytochemistry and quantitative immunogold electron microscopy to determine the localization and density of DG on podocyte membranes.

In MCN, the density of expression of α-DG was significantly reduced to 25% of controls, and that of β-DG to approximately 50%. Because immunogold labeling for α-DG was more intense than that of β-DG, the values obtained for the former may reflect the situation more accurately. The expression of β1-integrin was not influenced at all (Figure 1). Interestingly, steroid treatment of MCN patients apparently restored the expression of DG, along with the reformation of foot processes. This is consistent with the finding that the surface expression of the DG complex was upregulated by steroids in vitro in cultured muscle cells (37,38). Thus, it is possible that in MCN restoration of podocyte shape and glomerular filtration function by steroid therapy may involve upregulation of DG expression. It remains to be determined whether utrophin is altered in MCN.

The significance of apparently normal density of DG in FSGS is unclear, and it is possible that DG is irrelevant in the pathogenesis of FSGS. However, clustering of FSGS is unclear, and it is possible that DG is irrelevant in the altered in MCN.

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References


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