# Membranous Glomerulopathy With Spherules: An Uncommon Variant With Obscure Pathogenesis

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• ***Background:*** **Occasional case reports of membranous glomerulopathy described unique subepithelial accumulations of an unusual type of immune deposit composed of spherular structures. The identity of such structures as nuclear pores has been suggested, but not established.** ***Methods:*** **We identified a cohort of patients (n = 14, including 1 patient with disease recurrence in an allograft) who presented with nephrotic syndrome and had renal biopsy specimens with light and immunofluorescence microscopic findings characteristic of membranous glomerulopathy. These patients were distinguished by ultrastructural studies that showed glomerular capillary wall accumulations of subepithelial immune deposits composed of uniform spherular structures, while lacking the typical granular electron-dense deposits seen in membranous glomerulopathy. The molecular identity of these spherular structures as nuclear pores was tested by using immunofluorescence microscopy and immunohistochemistry with mouse monoclonal antinuclear pore antibodies (Covance, Princeton, NJ) and anti-Nuclear Pore-O-Linked Glycoprotein (Affinity BioReagents Inc, Golden, CO) antibodies.** ***Results:*** **Measurement of spherular structures by using high-magnification electron microscopy showed an average diameter of 84.5 nm, which correlated well with accepted diameters of nuclear pores (80 to 120 nm). Immunofluorescence microscopy and immunoperoxidase staining with both antibodies showed characteristic beaded staining of nuclear membranes of multiple cell types within normal control kidney, but no staining of immune-type deposits within glomerular basement membranes.** ***Conclusion:*** **These cases form a rare, but distinctive, morphological subclass of membranous glomerulopathy. The antigenic specificity of immune deposits in these cases remains elusive.** ***Am J Kidney Dis*** **47:983-992.**



**INDEX WORDS: Membranous glomerulopathy; spherules; nuclear pore.**

ANTIGENS THAT INCITE the immune response that leads to membranous glomerulopathy, one of the most common causes of nephrotic syndrome in adults, largely remain unknown. Although the majority of cases are idiopathic, secondary forms of membranous glomerulopathy may be caused by a number of disease processes, including infection, malignancy, autoimmune diseases, or exposure to certain therapeutic agents.[1-9] Although several antigens were proposed to have a role in the development of this disease, only a few were clearly established to be an etiologic agent of human disease.[10-12]

Occasional case reports in the English-language literature have illustrated subepithelial accumulations of an unusual type of spherular deposit in capillary walls of patients with membranous glomerulopathy. An intriguing insight into the nature of these particles came from a report by Dales and Wallace[13] of a case of membranous glomerulopathy with the ultrastructural finding of massive deposition of spherular organelles trapped predominately in the subepithelial space of glomerular capillary walls. Based on the morphological similarity of the structures to previously described nuclear pore complexes,[14] the investigators attempted to identify these glomerular structures as derivatives of such pores. They were able to show the presence of circulating antibodies in the affected patient against several cellular components, including discrete structures in nuclear membranes of cells consistent with an identity as nuclear pore. These findings suggested that the glomerular deposits might be composed largely of nuclear pore.

With this case report in mind, we identified a series of human renal biopsy specimens with light and immunofluorescence (IF) microscopic features characteristic of membranous glomerulopathy, in which deposits were composed of numerous and widespread subepithelial and/or intramembranous

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spherular particles similar to those described by Dales and Wallace[13] and others.[15,16] Because of the morphological similarity of these unusual deposits, we reasoned that these structures could be the consequence of a novel target antigen-antibody complex responsible for the development of membranous glomerulopathy, and this particular group of patients could represent a distinctive subset of patients with a common disease with a unique pathogenesis.

In this report, we describe clinical and pathological findings in a series of 14 patients with the distinctive pathological appearance of membranous glomerulopathy with spherular deposits and describe our attempts to further characterize these structures and confirm the hypothesis put forth by Dales and Wallace[13] that these curious particles constitute precipitated nuclear pores that have formed immune complexes with circulating antibodies.

## METHODS

With Institutional Review Board approval (04-0772-E01) for review of patient medical records, we searched the files of the Department of Pathology at the University of Washington Medical Center (Seattle, WA) for all renal biopsies accessioned from 1986 to 2004 (n = 10,149). We identified 864 cases of membranous glomerulopathy (8.5%). Seven of these cases (0.7% of membranous glomerulopathy cases) showed widespread atypical spherular ultrastructural deposits. These, together with 4 similar cases contributed by Dr Agnes Fogo from Vanderbilt University (Nashville, TN) and 3 cases contributed by Dr Donald Houghton from Oregon Health Sciences University (Portland, OR) form the substance of this report.

Demographic, clinical, and laboratory information for each patient were obtained at the time of kidney biopsy. Follow-up information was available for 7 patients. Renal insufficiency is defined as a serum creatinine level greater than 1.2 mg/dL (>106 μmol/L). Nephrotic syndrome is defined as 24-hour urinary protein excretion greater than 3 g/24 h, edema, and hypoalbuminemia (serum albumin <3.6 g/dL [<36 g/L]). Hypertension is defined as diastolic blood pressure of 90 mm Hg or greater.

All renal biopsies were processed by using standard techniques for light, IF, and electron microscopy (EM). Procedures for the University of Washington cases were as follows (similar procedures were used at Vanderbilt University and Oregon Health Sciences University). For light microscopic evaluation, 2-μm histological sections prepared from formalin-fixed paraffin-embedded tissue were stained with hematoxylin and eosin, periodic acid–Schiff reagent, and methenamine silver stain by following routine protocols. For IF studies, 3-μm cryostat sections were stained with fluorescein isothiocyanate–conjugated antihuman immunoglobulin G (IgG), IgM, IgA, C3, C1q, κ and λ light chain, fibrinogen, and albumin, as previously described.[17] The distribution and intensity of detectable staining was described and recorded by using a semiquantitive score of 1 to $4^+$.[17] Tissue for EM was fixed in half-strength Karnovsky solution, postfixed in 2% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in propylene oxide resin. Sections of 0.1 μm were stained with uranyl acetate and lead citrate and examined by using a Philips 410 electron microscope (Philips Export BV, Eindhoven, The Netherlands).

### *Antibodies*

Fluoresceinated antisera to deposited human IgG, IgA, IgM, C3, C1q, κ and λ light chain, and albumin were obtained from commercial sources (Diasoren Inc, Stillwater, MN), Monoclonal antibody m414 (Covance, Princeton, NJ) is a mouse antibody that recognizes the FG dipeptide (Phe-Gly) repeat motifs found in multiple proteins (nucleoporins) present in nuclear pores, structures found in all nuclear envelopes.[18]

A monoclonal mouse antibody to Nuclear Pore-O-Linked Glycoprotein (NP-O-LG; Affinity BioReagents Inc, Golden, CO) detects O-linked glycoproteins of the nuclear pore complex.[19]

For IF studies, cryostat sections were stained with m414 antibodies at 1:200 dilution and NP-O-LG at 1:100 dilution, as recommended, followed by secondary fluorescein isothiocyanate–conjugated antimouse antibody (Dako, Carpinteria, CA; 1:50 dilution). Control tissue included normal human kidney obtained from a nephrectomy performed for excision of a circumscribed tumor.

Immunohistochemistry (IHC) with both primary antibodies, used at the same dilution described, also was performed on formalin-fixed paraffin-embedded portions of biopsy specimens by using a standard avidin-biotin peroxidase protocol, as previously described.[20]

Both IF and IHC studies also were performed using anti-CD10 antibodies (mouse monoclonal; Novocastra Laboratories Ltd, Benton Lane, UK). Antibodies against CD10 (also known as common acute lymphoblastic leukemia antigen, nephrilysin, enkephalinase, and membrane metalloendopeptitase) recognize a neutral endopeptidase, a membrane-bound enzyme that can digest biologically active peptides.[21] CD10 normally is expressed on the surface of human podocytes, syncytiotrophoblastic cells, polymorphonuclear leukocytes, lymphoid progenitor cells, and many epithelial cells within nonlymphoid organs[8,22,23] and has been implicated in the pathogenesis of a subset of pediatric cases of membranous glomerulopathy.[11,12]

Serum samples from 3 patients (obtained at the time of the index biopsies) were used for Western blot analysis. Briefly, rat liver nuclei were prepared as previously described,[24] and nuclear pore complexes were isolated from these samples by using the zwitterionic detergent Empigen BB (Sigma-Aldrich Co, St Louis, MO).[25,26] Equal amounts of these fractions, both rat liver nuclei and purified nuclear pore complexes, were separated by means of electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel, transferred to nitrocellulose membranes, and blocked with 5% nonfat dry milk in tris buffered saline containing 0.5% Tween 20 (Sigma-Aldrich Co). These membranes were incubated overnight with either a 1:10 dilution of patient serum samples or a 1/2,000 dilution of mouse monoclonal antinuclear pore antibody (m414[18]) diluted in tris buffered saline-Tween containing 5% nonfat milk. Membranes were washed and incubated with goat antihuman or antimouse IgG horseradish-peroxidase conjugates for 60 minutes, washed, and

visualized by using enhanced chemiluminescence (Supersignal; Pierce, Rockford, IL).

## RESULTS

### *Clinical Data*

Clinical data for each patient are listed in Table 1. The group of 14 patients consisted of 7 women (50%) and 7 men (50%) with a mean age of 56 years (range, 34 to 75 years). All patients presented with proteinuria (mean urinary protein excretion, 7.6 g/24 h; range, 2 to 22 g/24 h); 8 patients had nephrotic syndrome and 6 patients had isolated proteinuria. Three patients had renal insufficiency with a mean serum creatinine level of 3.1 mg/dL (range, 1.3 to 6 mg/dL [274 μmol/L; range, 115 to 530 μmol/L]). Mean serum creatinine level of the remaining 10 patients was 1.0 mg/dL (range, 0.7 to 1.2 mg/dL [88 μmol/L; range, 62 to 106 μmol/L]). Significant concurrent diseases or conditions with potential renal impact included a long-standing history of systemic lupus erythematosus (1 patient), history of stem cell transplantation (1 patient), Sjögren syndrome (1 patient), 2-year history of diabetes mellitus (1 patient), and pregnancy (1 patient). All except 1 biopsy were of native kidneys. One patient with an allograft kidney biopsied 14 months after transplantation was found on retrospective review to have the atypical spherular deposits in a native kidney biopsy performed 20 years preceding kidney transplantation.

### *Pathological Findings*

*Light microscopy.* Numbers of glomeruli available for histological examination varied from 5 to 33 glomeruli/biopsy. Approximately 0 to 6 glomeruli/biopsy (0% to 18%) were globally sclerosed. In 2 cases, 10% of glomeruli showed additional features of segmental sclerosis. In all cases, there was mild to moderate thickening of glomerular capillary walls (Fig 1A and B). In the majority of cases (80%), there was formation of epimembranous projections of matrix ("spikes"), which were seen best on silver stain (Fig 1C). Spikes were focal in some cases and diffuse in others. In 6 cases, mesangial areas were mildly expanded by matrix and increased cellularity. All cases showed mild patchy interstitial fibrosis and tubular atrophy consistent with the amount of global glomerulosclerosis present in the given biopsy specimen. Mild intimal thickening of muscular arteries was present in 3 cases, and mild arteriolar hyalinosis, in 2 cases.

*Immunofluorescence.* IF microscopy for standard immune reactants (IgG, IgM, IgA, C3, C4, κ and λ light chains, fibrinogen, and albumin) was performed in 13 cases (in 1 case, no glomerulus was available for IF examination). Twelve of these 13 cases showed 2 to $3^+$ granular staining for IgG (Fig 1D) and C3 along glomerular capillary walls. Additionally, 3 cases showed staining for IgM (trace to $1^+$), and 2 cases, for both IgM (trace to $1^+$) and IgA (trace to $1^+$) in a similar distribution pattern. One patient with both IgA and IgG staining had a diagnosis of Sjögren syndrome. Staining for κ and λ light chains showed similar distribution, but less intense staining compared with IgG. No staining of tubules, interstitium, or renal vasculature was detected. In 1 case, the IF study was negative for all immune reactants tested.

*Electron microscopy.* Ultrastructural examination was performed in all 14 cases. On average, 2 glomeruli (range, 1 to 5 glomeruli) were examined per case. The most striking observation in all cases was diffuse thickening of glomerular capillary walls because of accumulation of aggregates of uniform hollow spherular structures. Particles were located in subepithelial (Fig 2A and C) and intramembranous (Fig 2B) locations in the majority of capillary loops. In 1 patient with systemic lupus, spherular particles also were present within mesangial areas. Particles contained central electron-lucent cores surrounded by an electron-dense outer layer and occasionally had a targetoid appearance (Fig 2D). These spherular structures had an average diameter of 84.5 nm (range, 84 to 90 nm). For comparison, published data on diameters of nuclear pores indicate an expected size range of 80 to 120 nm.[14,27-29] The granular electron-dense deposits typically seen in idiopathic membranous glomerulopathy and lupus membranous glomerulonephritis were not present in either capillary walls or mesangium. In all 13 cases, there was extensive effacement of epithelial-cell foot processes. Tubuloreticular inclusions were present in glomerular endothelial cells in the patient with Sjögren syndrome, but not the other cases, including the patient with systemic lupus.

### *Nuclear Pore Studies*

IF microscopy with m414 and NP-O-LG antibodies showed characteristic discrete beaded staining of nuclear membranes of multiple cell types within normal control kidney. Ten cases (with available

**Table 1. Clinical Features of Patients With Membranous Glomerulopathy With Unusual Deposits**

| Patient No. | Age (y)/ Sex | Clinical Presentation | Creatinine (mg/dL) | Proteinuria (g/24 h) | Serology | Pathological Diagnosis | Treatment | Follow-Up After Biopsy | Last Recorded Creatinine (mg/dL) | Last Recorded Proteinuria (g/24 h) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 53/F | Nephrotic syndrome, hypertension | 1.1 | 8.5 | NA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 2 | 63/M | Nephrotic syndrome, 2-y history of diabetes | 2.0 | >3.0 | NA | Membranous glomerulopathy | Steroids | 5 mo | On HD | NA |
| 3 | 62/F | Proteinuria, hypertension | 1.1 | 2.0 | ANA negative | Recurrent membranous glomerulopathy | Steroids, MMF, cyclosporine, ACE inhibitor | 2 y (25 y from the first biopsy of native kidney) | 1.8 | $1^{+}$(d) |
| Allograft biopsy 14 mo posttransplantation | | | | | | | | | | |
| 4 | 66/M | Systemic lupus, proteinuria | 1.0 | $3^{+}$(d) | NA | Lupus nephritis III + V (ISN/ RPS) | Steroids, cyclophosphamide | 4 mo | 1.1 | $3^{+}$(d) |
| 5 | 41/M | Nephrotic syndrome | 1.3 | 7.0 | ANA negative | Membranous glomerulopathy | Steroids, ACE inhibitor | 8 mo | 1.1 | 2.2 |
| 6 | 60/M | Nephrotic syndrome, s/p stem cell transplant for CLL | 6.0 | 22.0 | NA | Membranous glomerulopathy | Steroids | 4 mo | On HD | NA |
| 7 | 34/F | Proteinuria | 0.8 | 6.0 | ANA negative | Membranous glomerulopathy | Steroids, ACE inhibitor, ARB | 3 y | 1.2 | 13 |
| 8 | 68/F | Proteinuria | 1.0 | 6.3 | Elevated ANA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 9 | 55/M | Proteinuria, hematuria, hypertension | 0.7 | 8.9 | ANA negative | Membranous glomerulopathy | Steroids | 6 y | 1.1 | 4.6 |
| 10 | 39/F | 14 wk pregnant, nephrotic syndrome | 0.8 | 4.0 | NA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 11 | 75/F | Sjögren syndrome, hypertension, proteinuria | 1.4 | 2.5 | Elevated ANA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 12 | 39/M | Nephrotic syndrome | 1.2 | 15.0 | NA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 13 | 71/F | Proteinuria, hypertension, anemia | 1.1 | $4^{+}$(d) | NA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |
| 14 | 60/M | 10 y of seronegative RA, nephrotic syndrome | 1.2 | 6.0 | NA | Membranous glomerulopathy | NA | Lost to follow-up | NA | NA |

Abbreviations: s/p, status post; CLL, chronic leukocytic leukemia; ANA, antinuclear antibodies; NA, not available; ISN/RPS, International Society of Nephrology/Renal Pathology Society; RA, rheumatoid arthritis; MMF, mycophenolate mofetil; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HD, hemodialysis; (d), dipstick analysis.

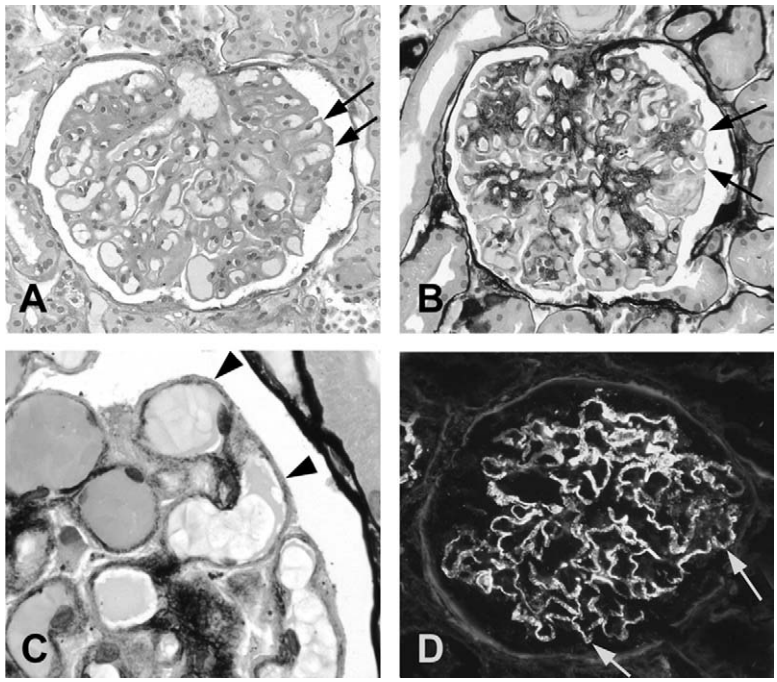


**Fig 1. Morphological findings in cases of membranous nephropathy with unusual deposits. (A, B) Normocellular glomeruli with diffusely thickened capillary walls (arrows). ([A] Periodic acid–Schiff stain; original magnification ×40; [B] methenamine silver–periodic acid–Schiff stain; original magnification ×40.) (C) High-power view of glomerular capillary wall showing thickening, irregularity, and focal spike formation (arrowheads). (Methenamine silver–periodic acid–Schiff stain; original magnification ×100 oil.) (D) Finely granular IF staining for IgG along glomerular capillary basement membranes (arrows). (Original magnification ×40.)**

frozen tissue) showed the same pattern of staining, but failed to stain immune deposits along glomerular capillary walls (Fig 3A and B). Immunolocalization studies with these same antibodies using an immunoperoxidase procedure on corresponding paraffin-embedded portions of the biopsy yielded identical results (Fig 3C and D).

IF and IHC studies performed on the same 10 cases with antibodies against CD10, an enzyme shown previously to be the autoantigen for a subset of pediatric patients with membranous glomerulopathy,[11,12] did not show staining of capillary wall immune deposits in our cases (Fig 3E). Positive internal control staining of podocytes and parietal epithelial cells was present in index cases and other controls, including 2 cases of a classic variant of focal and segmental glomerulosclerosis, 2 cases of a collapsing variant of focal and segmental glomerulosclerosis, and 2 cases of membranous glomerulopathy with typical granular deposits on EM examination.

Western blot studies using a preparation of purified nuclear pores and the 3 available patient serum samples were performed to test for the presence of circulating antinuclear pore antibodies, but immunoreactivity was not detected (data not shown).

*Clinical Outcome*

Information concerning clinical outcome was available for 7 patients (Table 1). Follow-up on the remaining patients was not obtainable. The follow-up period ranged from 4 months to 25 years. Three of 7 patients lost their kidney function. One patient, who also had a 2-year history of diabetes, progressed to end-stage kidney disease requiring hemodialysis treatment within 20 months after the index biopsy. A second patient who presented with renal failure (serum creatinine level, 6 mg/dL [530 $\mu$mol/L]) never regained kidney function and remained on hemodialysis therapy. The patient with involvement of a transplant kidney developed end-stage renal disease during a 20-year period after a diagnostic biopsy of the native kidney. Recurrence of the disease was detected 14 months posttransplantation, when the patient underwent an allograft biopsy for evaluation of proteinuria (urine protein excretion, 2.0 g/24 h; serum creatinine at that time, 1.1 mg/dL [97 $\mu$mol/L]; Fig 4A to D). During the next 2.5 years, the patient had persistent proteinuria and a slowly progressive increase in serum creatinine levels to 1.9 mg/dL (168 $\mu$mol/L).

Three of the remaining 7 patients experienced persistent proteinuria and a slow increase in serum creatinine level from 0.8 $\pm$ 0.08 to 1.1 $\pm$ 0.03

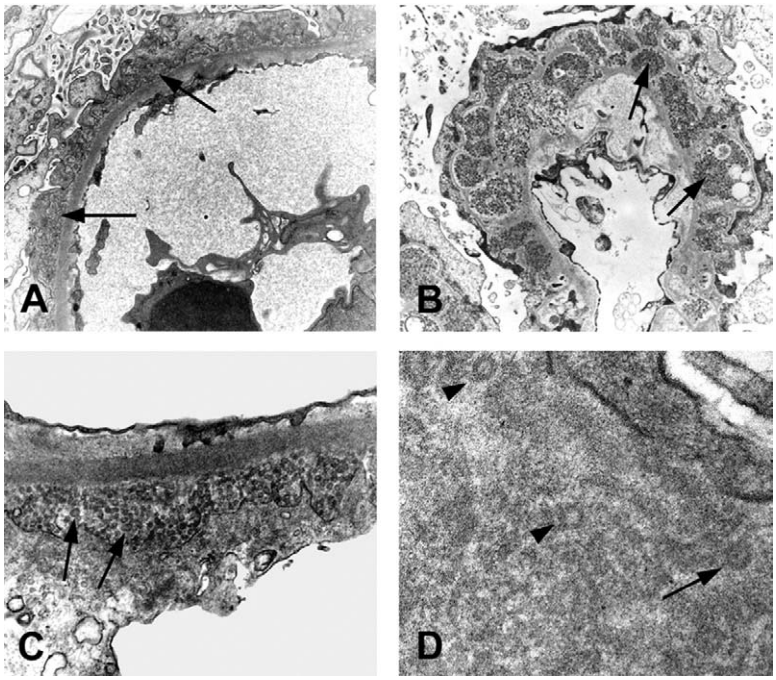


**Fig 2. EM findings. (A) Electron micrograph of a glomerular capillary wall thickened by accumulation of numerous subepithelial electron-dense deposits (arrows). (B) Glomerulus from a different case with diffuse thickening of the capillary wall by numerous intramembranous deposits of spherules (arrows). (C) High-power view of glomerular capillary wall with subepithelial round and oval particles. (d) Magnification of spherules shows central electron-lucent cores surrounded by an electron-dense outer layer (arrowheads) and occasional targetoid appearance (arrow). (Original magnification: [A, B] ×7,200; [C] ×12,000; [D] ×67,000.)**

mg/dL (71 ± 7.1 to 97 ± 3 μmol/L) during a period ranging from 4 months to 6 years. Two of these patients underwent follow-up kidney biopsies 2 and 6 years after the initial biopsies. Both biopsies showed more pronounced irregularity of glomerular capillary walls, marked increase in number of atypical deposits, and more prominent chronic tubulointerstitial injury. One of the follow-up biopsies showed segmental necrosis involving a minority (~10%) of sampled glomeruli. Serological studies for antineutrophil cytoplasmic antibodies and anti–glomerular basement membrane antibodies were negative.

One patient, who initially presented with a serum creatinine level of 1.3 mg/dL (115 μmol/L) and proteinuria with protein of 7 g/24 h, had a serum creatinine level of 1.1 mg/dL (97 μmol/L) and decrease in proteinuria to protein of 2.2 g/24 h after 8 months of follow-up.

Treatment protocols and follow-up were available for 7 patients. All 7 patients were administered steroids according to conventional management strategies for membranous glomerulopathy. Three of these patients also were administered angiotensin-converting enzyme inhibitors, and 1 patient additionally was administered an angiotensin receptor blocker. Despite treatment with steroids, improvement in renal function occurred in only 1 patient (serum creatinine improved from 1.3 to 1.1 mg/dL [115 to 97 μmol/L]). One patient's serum creatinine level remained stable at 1 to 1.1 mg/dL (88.4 to 97.2 μmol/L). The remaining 5 patients experienced worsening of kidney function: 2 patients developed hemodialysis-dependent end-stage renal failure and 3 patients had an increase in serum creatinine level from an average of 0.87 to 1.37 mg/dL (77 to 121 μmol/L), all within an approximately 2-year follow-up period. The decrease in proteinuria in a single patient (protein from 7 to 2.2 g/24 h) correlated with the improvement in serum creatinine level, whereas in the remaining patients, proteinuria either persisted at the same level as at the time of biopsy or increased (in 1 patient, from 6 to 13 g/24 h of protein). The addition of more potent immunosuppressive drugs, such as mycophenolate mofetil and cyclosporine in the case of the allograft kidney, did not stop the progression of kidney insufficiency (from 1.1 to 1.8 mg/dL [97 to 159 μmol/L] during 2.5 years). The addition of cyclophosphamide therapy for the patient with systemic lupus resulted in stable renal function within a short follow-up period of 4 months. These results are not dissimilar

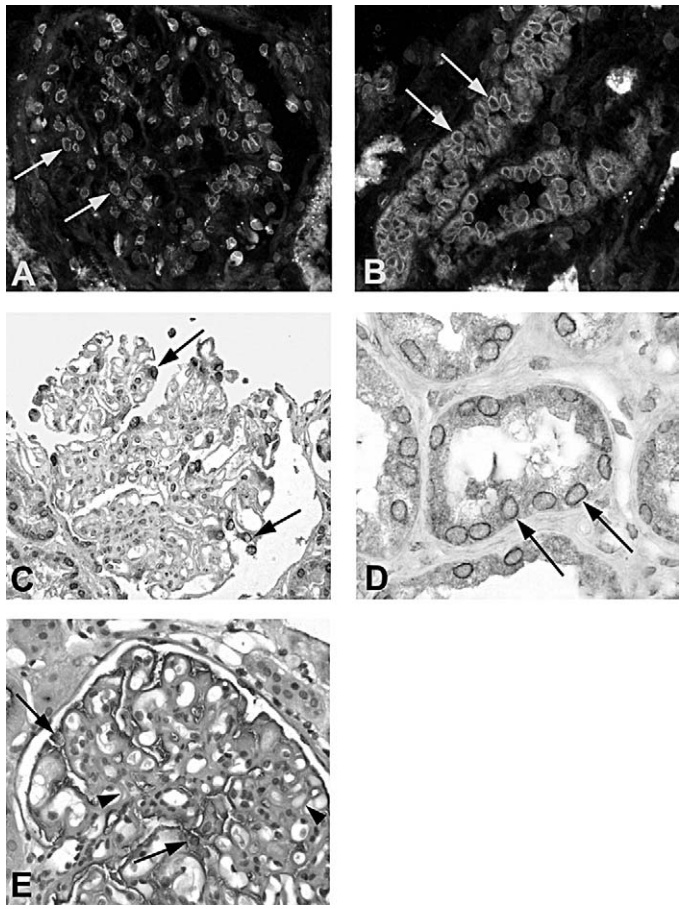


**Fig 3. Staining with antinuclear pore antibodies and anti-CD10. (A, B) IF staining with anti-m414 nuclear pore antibodies shows finely granular staining of nuclear membranes of different cell types (arrows; [A], glomerulus, [B], tubules), but without staining of glomerular basement membrane deposits. (C, D) Immunoperoxidase technique with antinuclear pore antibodies of a glomerulus and tubules with similar results (arrows). (E) Immunoproxidase staining with anti-CD10 antibodies of glomerular visceral epithelial cells (arrows), but not basement membrane deposits (arrowheads).**

from those of most patients receiving similar therapeutic regimens.

## DISCUSSION

Membranous glomerulopathy is characterized by the presence of immune complexes within glomerular capillary walls, shown best by using IF and EM. Ultrastructurally, typical immune complexes in membranous glomerulopathy are discrete, granular, electron-dense deposits present predominately on the subepithelial surface of the glomerular capillary basement membrane.

Although membranous glomerulopathy is among the most common causes of nephrotic syndrome, the antigenic substrate(s) for immune complexes that cause this disease in humans remain unknown. The Heymann model of nephritis in rats suggests the likely involvement of target antigens present in glomerular visceral epithelial cells in many cases, although it is possible that exogenously planted antigens also may be pathogenic in some cases. Many reports claimed to identify putative antigens, including DNA, thyroglobulin, tumor-associated antigens, renal tubular epithelial antigens, and such viral proteins as hepatitis B virus[10,30] and, most recently, neutral endopeptidase[11,12] in cases of membranous glomerulopathy. However, localization of such proteins in affected glomeruli does not necessarily confirm their pathogenic role as target antigens. Except in very rare cases, such as those involving neutral endopeptidase,[11,12] existing data for human membranous glomerulopathy do not clearly establish which antigens are targeted in the subepithelial deposits.

In our cases, the unusual deposits were abundant, present diffusely in the majority of glomerular capillary walls, and all except 1 case had a discrete pattern of deposition of immune reactants similar to that seen in membranous glomerulopathy. We hypothesized that the unique ultrastructural morphological characteristics of these structures represented the substrate for a novel target antigen-

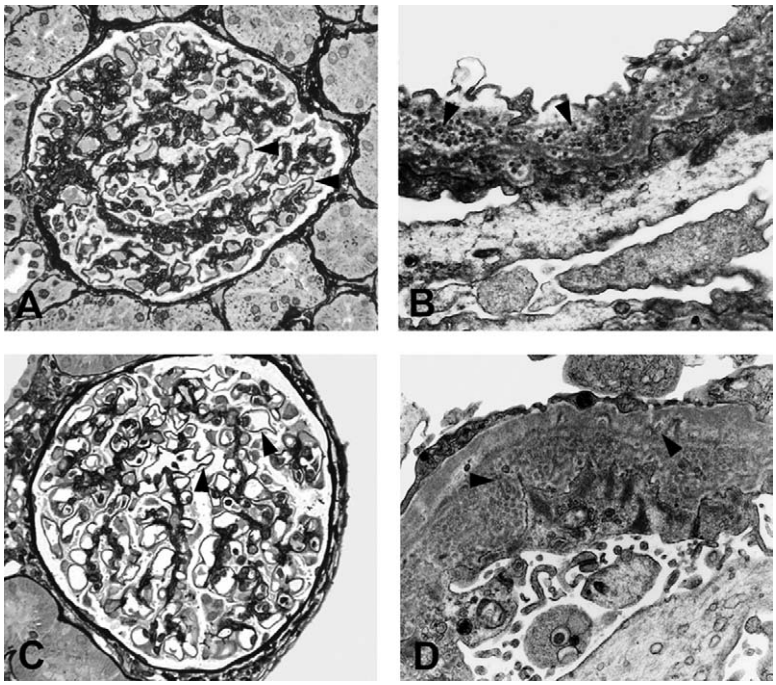


**Fig 4. Case of recurrent membranous nephropathy in a kidney allograft. (A, B) Biopsy of native kidney showing thickening of capillary walls (arrowheads) and spherular deposits on EM (arrowheads). ([A] Methenamine silver–periodic acid–Schiff stain; original magnification ×40; [B] ×7,200.) (C, D) Biopsy specimen from the same patient's allograft kidney 14 months after transplantation with similar features on light microscopy (arrowheads) and EM (arrowheads). ([C] Methenamine silver–periodic acid–Schiff stain; original magnification: [C] ×40, [D] ×7,200.)**

antibody complex responsible for the development of membranous glomerulopathy, and hence this particular group of patients represents a distinctive subset of membranous glomerulopathy with a unique pathogenesis. Support for this hypothesis is provided by the development of recurrent disease in an allograft kidney with ultrastructural features identical to the native kidney. This clinical observation indicates that this particular manifestation of membranous glomerulopathy represents a specific host response to an as yet undefined antigen that is not unique to the patient's own kidney.

The report by Dales and Wallace[13] of a case of membranous glomerulopathy with unusual spherical deposits was followed by a few case reports with similar findings,[31,32] but none had information that would help us understand the basis for the unique morphological characteristics of the deposits. Spherular deposits also were reported in patients with other glomerular immune complex deposition diseases,[16] but we have not encountered prominent spherular deposits in these other entities in our own biopsy service or in surveys by others of the ultrastructural pathological states of glomerular diseases.

Dales and Wallace[13] identified circular structures similar to nuclear pores in sedimented plasma from a patient with membranous glomerulopathy and also showed that this patient's serum contained autoantibodies to nuclear components, including structures consistent with an identity as nuclear pores. Based on these findings, Dales and Wallace[13] postulated that nuclear pores might circulate as immune complexes and become trapped within glomerular capillary walls. Their conclusion that the peculiar deposits may be nuclear pores was rejected by Barry and Rennke,[15] who considered the shape of the spherular deposits to not correspond to the cylindrical shape of nuclear pores and considered that size constraints would make it unlikely that such structures could pass from the circulation through the glomerular endothelial fenestrae and lodge in capillary walls.

Nuclear pores are ubiquitous among eukaryotic cells and serve as portals between the nucleus and cytoplasm for regulated transport of a variety of molecules.[33,34] By using IF and IHC with 2 different commercially available antibodies to nuclear pore constituents, we found no evidence to support the hypothesis that spherical deposits in our cases were nuclear pores. However, the possibility that spherular deposits in our cases are derived from nuclear pores cannot be completely excluded because in certain conditions, the antigenic epitopes may be hidden and therefore not available for detection with staining techniques requiring the

antigen-antibody reaction. Because of a paucity of tissue remaining in the blocks after the standard and additional IF and IHC staining for nuclear pore antibodies, we were not able to perform antibody elutions to further examine for the presence of nuclear pore antigens.

The basis for the absence of IF staining for all immunoglobulins tested in 1 of the cases is not readily apparent. We speculate, but cannot prove, that this may be caused by technical reasons related to tissue preservation before accessioning the biopsy specimen in the laboratory, such as might occur if the biopsy cores had inadvertently been placed in formalin before submission for IF studies. This finding represents a true biological phenomenon and we doubt there is a nonimmune basis for glomerular deposits in this case.

We also were unable to show circulating antibodies to nuclear pore complexes in sera from affected patients from whom it could be obtained. We were stimulated by the recent publication by Debiec et al,[11] in which a specific target antigen, neutral endopeptidase, elicited membranous nephropathy with intriguingly ultrastructural demonstration of spherular deposits similar in appearance to those reported in our series. We therefore also tested the possibility that in our cases, immune complexes contained neutral endopeptidase. IHC tests for the presence of neutral endopeptidase (CD10) in spherular deposits by using 2 different antibody probes also were unsuccessful.

We conclude that our cases form a morphologically distinct subset of membranous glomerulopathy caused by a specific antigen of unknown origin. Overall, the clinical course of patients with spherular deposits does not appear substantially different from that of generic cases of membranous glomerulopathy. No clear conclusions could be drawn from available data regarding treatment protocols in these patients. It would be reasonable to deduce that management of these patients should follow standard treatment protocols for membranous glomerulopathy.

In summary, we present a morphologically unique subset of membranous glomerulopathy characterized ultrastructurally by the presence of spherular immune deposits of undefined origin. The common ultrastructural appearance of immune deposits and recurrence in an allograft kidney suggests that the membranous glomerulopathy induced in these patients results from a common autoantigen. Evidence against the possibility that this antigen represents part of the nuclear pore complex or the neutral endopeptidase moiety has been presented, and the identity of the antigen remains elusive. No specific associations could be made on the basis of the clinical histories, although recurrence in an allograft kidney implicates a target antigen that is widely distributed in the human population. Because of a limited number of patients with adequate follow-up, we are unable to clearly predict the outcome of patients with this uncommon disease.

The origin of the spherical structures remains an unsolved mystery.

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