Podocyte Infolding Glomerulopathy (PIG) is a recently described pathologic entity characterized by diffuse podocyte infolding into the glomerular basement membrane (GBM) associated with ultrastructurally demonstrable microspherular aggregates. The clinical features, significance, and pathogenesis of this condition are still not well delineated because only a few cases have been documented to date, all from Japan. We report a case of PIG associated with undifferentiated connective tissue disease in an Indian woman who presented with nephrotic syndrome while undergoing treatment for an autoimmune disorder. Ultrastructural analysis of the kidney biopsy specimen revealed unusual subepithelial aggregates of microspherules admixed with few microtubules alongside extensive infolding of podocyte foot processes into the underlying GBMs. Characteristic clustering of these microparticles near the invaginated tips of podocyte foot processes in the GBM was observed on transmission electron microscopy. The patient’s clinical condition responded favorably to immunosuppressive therapy. The clinical, light microscopic, and diagnostic electron microscopic features of this condition are highlighted in this report in an attempt to contribute some insights into the possible pathogenetic mechanisms of this obscure entity.

Introduction
Podocyte infolding glomerulopathy (PIG) has been recently proposed as a new pathologic entity based on the unique ultrastructural finding of podocyte infolding and invagination into the glomerular basement membranes (GBMs) manifesting as microspherules and microtubules visualized on electron microscopy (EM). Since the first report of this peculiar EM finding in 2002, it was thought to be a variant of membranous nephropathy (MN) exhibiting annular subepithelial deposits. It is only in the past few years that PIG has begun to be recognized as a possible new and distinct disease entity. We report a case from India fulfilling the diagnostic criteria of PIG in a patient presenting with nephrotic syndrome while being treated for undifferentiated connective tissue disease. Because there have been only a few reports of this entity in the literature confined geographically to Japan, there is a need to share information about this entity from all parts of the world to characterize the spectrum of this glomerulopathy.

Case Report
A 45-year-old Indian woman with a diagnosis of undifferentiated connective tissue disease was prescribed methotrexate and hydroxychloroquine for symmetric non-deforming inflammatory polyarthritis of 1 year’s duration. Four months later, she presented with bilateral pedal edema and generalized malaise of 2 months’ duration. There was no relevant past or family history of autoimmune diseases.

On physical examination, the patient had pallor and bilateral pitting edema. Blood pressure was recorded as 130/80 mm Hg. Laboratory data were as follows: serum creatinine, 1.65 mg/dL; serum albumin, 1.5 g/dL; hemoglobin, 7.2 g/dL; erythrocyte sedimentation rate, 73 mm/h; tests for hepatitis B and C and human immunodeficiency (HIV) viruses gave negative results. Additional testing gave the following results: serum C3, 64.9 (reference range, 90-180) mg/dL; C4, 22.6 (reference range, 10-40) mg/dL; glycated hemoglobin, 4.8%; antinuclear antibody was detected (3+; homogeneous); double-stranded DNA, 108 (reference range, <100) IU/mL; anti-SSA by enzyme-linked immunosorbent assay, 110 RU/mL (reference range, <20 RU/mL); and anti-SSB by enzyme-linked immunosorbent assay, 32 RU/mL (reference range, <20 RU/mL). Serum electrophoresis showed decreases in total protein and albumin concentrations. Urinalysis showed blood (2+), protein (4+), and 10 white and 20 red blood cells per high-power field. Urine protein excretion was 5.8 g/d, with urinary protein-creatinine ratio of 9.89.

Kidney biopsy revealed a total of 15 glomeruli, 2 of which showed focal segmental mesangial proliferation with mild capillary wall thickening and focal segmental sclerosis (Figs 1A and S1A). Immunofluorescence (IF) showed positivity for immunoglobulin G (IgG; 1+) and C3 (trace) on capillary walls. Immunochemical staining for phospholipase A2 receptor (PLA2R) antigen yielded negative results. EM showed irregular thickening of GBMs with peculiar subepithelial clusters of microspherules (Figs 1B-D and 2A-C). Diffuse podocyte foot-process effacement associated with microvillous transformation and podocyte cytoplasmic vacuoles indicative of podocyte injury were noted (Fig S1C and D). Additionally, there was global infolding of podocyte cell processes into the underlying thickened GBMs, remarkable for its diffuse involvement and proximity of the invaginations to the...
microspherular clusters (Figs 2B-D and S1B and C). There were no discrete electron-dense deposits in the GBM or mesangium. A final diagnosis of PIG was rendered based on these findings.

The patient was treated with high-dose prednisolone and mycophenolate mofetil for 6 weeks, and a slow tapering schedule of steroids was planned. In view of aggressive disease, she was also given a single dose of 1 g of rituximab. On follow-up after 7 months, there was marked improvement in kidney function (serum creatinine, 0.98 mg/dL) and slow resolution of proteinuria (urine protein excretion of 1 g/d; urinary protein-creatinine ratio, 2.16).

Discussion

Although it is uncertain whether PIG is a new disease entity or a transient morphologic phenomenon, it is worthwhile to discuss the salient features of this case to shed more light on our understanding of this entity.

Light microscopic examination of the biopsy specimen in this case showed mild thickening of capillary walls with focal segmental sclerosis. In conjunction with IF studies that showed positivity for IgG (1+) and C3 (trace) on peripheral capillary loops, a preliminary diagnosis of MN was made. However, EM revealed subepithelial clusters of microspheres measuring 50 to 70 nm, some having distinct double membranes (Fig 2D, inset). Although the microspherular substructure was not readily evident at lower EM magnifications (Fig 1B), higher magnifications revealed diffuse microparticular substructure involving all the GBM “deposits” (Figs 1C and D, 2A-C, and S1B and C). In this context, it became necessary to differentiate this case from a variant subtype of MN and entertain the possibility of a separate pathologic entity with distinct EM features.

Figure 1. Light and electron microscopy (EM) of kidney biopsy specimen. (A) Glomerulus shows mild mesangial proliferation with segmental sclerosis and mild prominence of podocytes (arrows) (periodic acid–Schiff; original magnification, ×200). (B) Irregularly thickened glomerular capillary walls with subepithelial “deposits” (arrow) and intervening “spikes” reminiscent of membranous nephropathy (MN) stage II. The microspherular nature of deposits is not well appreciated at this magnification (transmission EM; original magnification, ×6,000). (C) Subepithelial microspherular clusters (arrow) with distribution pattern of stage II MN, extensive podocyte foot-process effacement, and infolding into the glomerular basement membrane (GBM; transmission EM; original magnification, ×8,200). (D) Microspherular clusters (arrow) surrounded and separated by intervening GBMs resembling MN stage III pattern (transmission EM; original magnification, ×9,900). Abbreviations: E, endothelial cell; P, podocyte; RBC, red blood cell.
Microstructures in the GBM are typically not found in classic MN, aside from occasional granular and membranous debris in stage III MN and in rare cases of hepatitis B virus–associated glomerulonephritis.4 Our case did not show immune-type deposits on EM, ruling out consideration of MN as the primary lesion, and the patient was not hepatitis B virus positive. Kowalewska et al have reported 14 cases of MN with microspherular GBM clusters exhibiting targetoid appearance, morphologically resembling nuclear pore complexes, that did not stain with antinuclear pore antibody and anti-neutralendopeptidase (NEP) (CD10) antibody.11 A case of antenatal MN induced by anti-NEP antibodies has also been described as showing microspherules on ultrastructural evaluation.12 Although the absence of immune deposits augured against a diagnosis of MN, the pathognomonic ultrastructural findings observed in our case were the widespread extent of podocyte infolding into underlying GBMs, proximity of the microspherular clusters to the invaginated tips of podocyte foot processes, and focal evidence of vacuolar degeneration of infolded podocyte tips (Fig 2D). Taken together, these findings favored PIG as the primary pathology.

The pathogenesis of PIG remains obscure. The term microspherical particle is used to describe membrane-bound vesicles with a lucent core, generally 40 to 100 nm in diameter, which are found in certain extracellular locations in a variety of human or animal tissues. In most of these situations, the distribution of microspherules within the GBM is focal and segmental, prompting
etiolologic considerations of viral infections, glomerular injury, and crystalline bodies. PIG lesions are global and diffuse in distribution and require delineation from such localized occurrences of microspherules.

Immune EM analysis of the glomerular lesions of PIG has demonstrated complement C5b-C9 and vimentin in the extracellular organized structures in the GBM, indicative of either podocyte or endothelial cell origin of these particles. In the largest study of PIG to date, which comprised 25 patients, a high incidence of autoimmune disease was observed, with improvement in proteinuria on corticosteroid therapy. The present case of PIG occurred in a patient with underlying autoimmune pathology (undifferentiated connective tissue disease) and hypercomplementemia, was weakly positive for immunoglobulins by IF without corroborative electron-dense deposits on EM, and showed clinical improvement in kidney function and proteinuria with immunosuppressive therapy. These findings provide support for the hypothesis of immune abnormalities, with hyperactivation of the complement pathway playing a key role in the pathogenesis of PIG. It is possible to speculate that failure to detect discrete electron-dense deposits on EM may pertain to the uniqueness of the immune complexes formed, which may not condense appropriately for deposit formation, in addition to increased sensitivity of IF for detecting scarce amounts of immune deposits.

Addressing the question of whether podocytes or endothelial cells give rise to the microspherules, features suggestive of podocyte injury, such as extensive foot-process effacement, microvillus transformation, and cytoplasmic vacuolation, have been documented in most cases of PIG and were present in our case also (Fig S1C and D). Moreover, the characteristic subepithelial location of the microparticles and clustering around infolded podocyte foot processes with focal evidence of vacuolar degeneration of invaginated podocyte tips suggest that disintegration of injured podocyte cell processes results in the formation of microspherules that migrate into the GBM. Further studies to evaluate the expression of podocyte-specific proteins such as podocin, glomerular epithelial protein 1, α-actinin 4, synaptopodin, and podocalyxin are required in a larger number of cases before final incrimination of the podocyte as the cell of origin of these ultrastructures.

In addition to podocyte injury, it is also feasible that abnormalities in the GBM may play a facilitatory role in the development of PIG. This is supported by the well-known finding of ultrastructurally similar microparticles trapped in the “basket weave” GBMs of patients with hereditary nephritis, albeit in scattered and limited amounts. The notion of GBM injury or abnormality contributing to easy trapping of degenerated podocyte cell processes and their subsequent migration within the GBM in patients with PIG merits consideration.

Irrespective of whether PIG is a novel disease entity, it is worthwhile to increase awareness of this entity in consideration of the uniformly reported favorable response to prompt initiation of immunosuppressive therapy. Although considerable attention is being garnered by this rare and peculiar “glomerulopathy” currently, careful EM studies of all kidney biopsies from patients with proteinuria and underlying autoimmune disorders may be recommended to discover more cases, elucidate the clinicopathologic profile of PIG, and formulate management strategies of this entity for the future.

Supplementary Material

Figure S1: Microscopy images of segmental proliferation of podocytes with mild increase in mesangial cells; podocyte foot process infolding with adjacent aggregation of microparticles in the GBM; microvillous transformation of podocytes with infolding of podocyte foot process into underlying GBM surrounded by microspherular and microtubular clusters; crowding of podocytes with cytoplasmic vacuolation and microvillous transformation indicative of possible insult to podocytes.

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