

Immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: an unusual cause of proteinuria in infancy

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Abstract We report on a 6-month-old child presenting with chronic diarrhea, failure to thrive, eczema, autoimmune hemolytic anemia (AIHA), insulin-dependent diabetes mellitus (IDDM), hypoalbuminemia, and proteinuria. Renal biopsy showed membranous glomerulonephritis. A diagnosis of Immunodysregulation, polyendocrinopathy, enteropathy, x-linked (IPEX) syndrome was subsequently confirmed by DNA analysis, which demonstrated the presence of a mutation in exon 2 of the *FOXP3* gene (303–304 del TT). Proteinuria secondary to membranous glomerulonephritis is a novel feature of IPEX syndrome. Membranous glomerulonephritis went into remission after the patient had received hematopoietic stem cell transplantation (HSCT).

Keywords IPEX syndrome · Proteinuria · Nephrotic syndrome · Membranous glomerulonephritis · Autoimmunity · FOXP3

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Introduction

Proteinuria in infancy is rare and may occur due to congenital nephrotic syndrome, diffuse mesangial sclerosis, Denys–Drash syndrome, congenital infections, such as cytomegalovirus (CMV), Epstein–Barr virus (EBV) and syphilis, and other uncommon disorders such as Galloway–Mowat syndrome and rare disorders of the glomerular basement membrane [1–5]. Rarely, idiopathic minimal change and focal segmental glomerulosclerosis cause nephrotic syndrome in late infancy [6]. Here, we describe a 6-month-old boy who presented with failure to thrive, eczema, diarrhea, metabolic acidosis, hyperglycemia, hypoalbuminemia, and proteinuria. Renal biopsy showed membranous glomerulonephritis. A diagnosis of Immunodysregulation, polyendocrinopathy, enteropathy, x-linked (IPEX) syndrome was subsequently confirmed by DNA mutational analysis. Several of his systemic features and proteinuria resolved after an unrelated donor hematopoietic stem cell transplantation (HSCT), as reported previously [7].

Case report

A 6-month-old African–American male infant was hospitalized with a 2-month history of significant weight loss, vomiting, and diarrhea. He was born at 41 weeks' gestation, his birth weight was 4.48 kg (80th percentile) and his length was 54.6 cm (77th percentile). There were no complications during pregnancy or delivery, and the results of his newborn screening test were normal. The review of his systems was remarkable for eczema, that had been treated by his primary care physician since early infancy, and a

history of two visits to the emergency room for vomiting. His family history was non-contributory. He was the first child of non-consanguineous African–American parents.

Physical examination at admission revealed a malnourished, pale infant with partial alopecia, facial puffiness, and edema of the hands and feet. He weighed 5.9 kg (<1st percentile), and his height was 65 cm (4th percentile). He was afebrile and had a heart rate of 147 beats per minute, a respiratory rate of 40 breaths per minute, and a blood pressure of 78/50 mmHg. He had multiple, enlarged, non-tender lymph nodes in his neck, axillae and inguinal areas. Skin examination showed the presence of multiple eczematous lesions. The findings of the rest of his physical examination were unremarkable.

Laboratory findings at admission showed hemoglobin of 8.3 gm/dl and a normal white blood cell and platelet count. Presence of an elevated reticulocyte count, low serum levels of haptoglobin, warm and cold autoantibodies, a positive result of a direct Coombs test, and peripheral blood smear showing hemolysis, suggested the diagnosis of autoimmune hemolytic anemia (AIHA). Serum chemical analyses showed increased anion gap metabolic acidosis that was secondary to lactic acidosis and ketoacidosis, as determined by serum ion chromatography. The child had normal renal function (serum creatinine 0.2 mg/dl), hypoalbuminemia (albumin 1.9 gm/dl), mild indirect hyperbilirubinemia, and mildly elevated levels of gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT). The presence of hyperglycemia (glucose 245 mg/dl), a low serum C-peptide level at <0.5 ng/ml (normal 0.8–3.1 ng/ml), and elevated glutamic acid decarboxylase (GAD) autoantibody at 115.9 units/ml (normal 0–1.5 units/ml) suggested immune-mediated pancreatic damage. He had low serum free T4 levels at 0.5 ng/dl (normal 0.8–2 ng/dl), indicating hypothyroidism. Stool studies were negative for infectious organisms but showed evidence of fat malabsorption. Biopsies from esophagus, stomach and duodenum showed infiltration with acute and chronic inflammatory cells.

Urinalysis showed a specific gravity of 1.021, pH 5.5, 4+ protein, glucose 500 mg/dl, large blood, and trace ketones. Microscopy showed four white blood cells (WBCs), 12 red blood cells (RBCs), multiple hyaline and occasional granular casts. The urine protein-to-creatinine ratio was elevated at 4, and urine β_2 microglobulin was elevated at 7,139 (0–300) μ g/l. Urine protein electrophoresis showed the presence of 74% albumin, and 8% α_1 , 3.2% α_2 , 5% β and 10% γ globulins in the urine. Serum IgG and IgA levels were elevated at 867 mg/dl (normal 140–533 mg/dl) and 78 mg/dl (normal 0–40 mg/dl), respectively, and IgM level was normal. Serum IgE was elevated at 1,564 (<20) IU/ml, and serum C3 and C4 levels were normal. Serology findings for

hepatitis viruses, syphilis, and CMV and EBV infections were negative, ruling out secondary causes of proteinuria in this age.

Renal biopsy showed thickening of the glomerular basement membrane, prominent visceral epithelial cells, and evidence of mild tubulointerstitial fibrosis on light microscopy. Jones silver stain showed spikes on the basement membrane. Immunofluorescence examination showed 3+ granular IgG deposits, 2+ C3, and 1+ C1q along the glomerular capillary walls, and few IgG deposits around the tubular basement membrane. Electron microscopy showed prominent subepithelial electron-dense deposits consistent with immune complex-mediated membranous glomerulonephritis (Fig. 1).

Based on the presence of AIHA, hypothyroidism, insulin-dependent diabetes mellitus (IDDM), chronic diarrhea, and eczema, a presumptive diagnosis of IPEX syndrome was made. Immune complex-mediated membranous glomerulonephritis was considered a novel feature of IPEX syndrome. The diagnosis was subsequently confirmed by mutational analysis, which demonstrated the presence of a mutation in exon 2 of the *FoxP3* gene (303–304 del TT).

The patient was initially treated with cyclosporine and prednisone and 4 weekly doses of rituximab. Although his AIHA responded to therapy, the IDDM, chronic diarrhea, hypothyroidism and proteinuria persisted. Three months later, he underwent an unrelated-donor cord-blood transplantation [7]. However, he promptly rejected his graft and

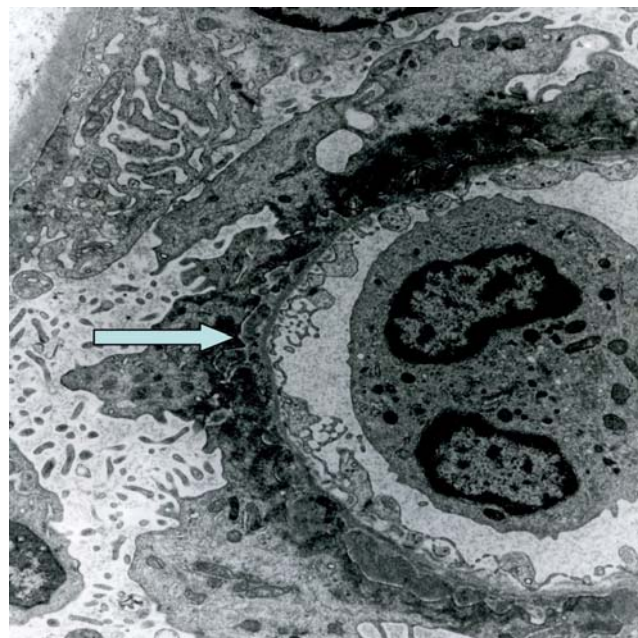


Fig. 1 Electron micrograph showing sub-epithelial electron-dense deposits (arrow)

reconstituted his hematopoietic system with all recipient-derived cells. He had several post-transplant complications, that included ongoing diarrhea, and recurrent *Clostridium difficile* enterocolitis, poorly controlled diabetes, hypertension, recurrent fevers and bacteremia, deep venous thrombosis of his arm and leg and issues with poor nutrition.

He underwent a second unrelated-donor HSCT at the age of 15 months [7]. This time, he engrafted with full donor hematopoietic chimerism and was successfully weaned off all immune suppressive medications. At his last follow-up, 30 months after his second HSCT, he had mild hypothyroidism and IDDM. His membranous nephropathy has been in remission since 6 months after his second transplant. At his last follow-up, his serum creatinine was 0.3 mg/dl, serum albumin was 4.2 g/dl, IgG, IgA and IgM levels were normal, and urinalysis yielded normal findings, with no evidence of proteinuria.

Discussion

IPEX syndrome is a rare, recessive disorder resulting in aggressive autoimmunity and early death [8, 9]. Protean features include early onset type 1 diabetes mellitus, severe enteropathy, eczema, anemia, thrombocytopenia, and hypothyroidism [8–11]. Rarely, autoimmune nephropathy has been reported in children with clinical features resembling IPEX syndrome [12–14]. However, nephropathy has not been reported in children with confirmed molecular diagnosis of IPEX syndrome. Wildin et al. summarized the data of 56 children with clinical features of IPEX syndrome in 2002 [13]. Of these, three were noted to have had renal involvement. Clinical manifestations included proteinuria, proximal tubular dysfunction, and progressive renal insufficiency. Renal pathology in these children showed focal tubular atrophy and tubulointerstitial nephritis in two children and membranous glomerulopathy with irregular granular immune deposits in glomeruli and tubular basement membranes in one child [14]. Our patient showed nephrotic range proteinuria and also had elevated urinary β_2 microglobulin levels indicative of proximal tubular dysfunction. Other causes of proteinuria in infancy were ruled out. Renal pathology in this child showed immune complex-mediated membranous glomerulonephritis, focal tubular atrophy, and peritubular immune deposits. Autoantibodies against renal epithelial cells may be responsible for this pathologic lesion. Specific antibodies against a 75 kDa antigen in intestinal and renal epithelial cells have been identified in an IPEX like patient with renal and intestinal involvement [15]. The renal component of IPEX syndrome can be reversed with bone marrow transplantation, as seen in this patient.

Mutations in the *FOXP3* gene in Xp11.23, encoding a protein called scurfin, were recently identified in several patients, thus establishing a genetic etiology [8, 16]. The scurfy mouse is a natural mutant with manifestations resembling IPEX. Scurfy mice show X-linked recessive inheritance, scaly skin, runting, progressive anemia, thrombocytopenia, leukocytosis, lymphadenopathy, hypogonadism, infection, diarrhea, gastrointestinal bleeding, cachexia, and death by 3–4 weeks of age [17].

The functions of normal scurfin and the molecular mechanisms by which mutated versions cause disease are not wholly known. Scurfin binds to DNA in the interleukin (IL)-2 promoter and granulocyte macrophage colony stimulating factor enhancer near nuclear factor of activated T cells, and inhibits transcription of these genes, resulting in reduced expression of these cytokines in activated CD4+ T cells [18]. The *FOXP3* mutation thus results in decreased numbers of functional regulatory T-cells which are unable to regulate immune responses. Pathological evidence supports a hyperactive immune dysregulated state that results in destruction or compromise of essential organs [13]. Studies in scurfy mice support this theory, proposing that abnormal T-cell reactivity can play a role in autoimmunity and cytokine excess. Scurfy mice have shown mixed perivascular infiltrates of lymphocytes, macrophages, and granulocytes in the kidney [17].

If untreated, most children will die by 24 months of age. Death in IPEX syndrome can occur from overwhelming sepsis, hemorrhage, intractable diarrhea, and the complications of diabetes [13]. Aggressive immune suppressive therapy has been shown to prolong life, but it poses a risk of opportunistic infections and the long-term toxicity of immune suppressive medications [19]. HSCT has been shown to be beneficial in a few reported cases of children with IPEX syndrome and can result in the reversal of the autoimmune manifestations of the disease [7, 20, 21]. Our child had mild residual hypothyroidism and IDDM after transplantation, indicating that some irreversible organ damage may have occurred prior to HSCT. Early HSCT holds the promise of nearly complete reversal of end-organ damage.

In conclusion, proteinuria can rarely occur in patients with IPEX syndrome. Failure to thrive, diarrhea and hypothyroidism can occur secondary to massive proteinuria in infancy, and eczema may be seen in children with nephrotic syndrome; however, AIHA and hyperglycemia are not seen [1, 22]. Therefore, a diagnosis of IPEX syndrome should be suspected in a male child presenting with proteinuria in association with intractable diarrhea, eczema, AIHA and IDDM. Early HSCT should be considered in IPEX syndrome to prevent the end-organ damage caused by immune complex deposition.

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