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CASE REPORT

Abrogation of nephrotic proteinuria by rituximab treatment in a renal transplant patient with relapsed focal segmental glomerulosclerosis

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Summary

Relapse of focal segmental glomerulosclerosis (FSGS) after renal transplantation is 20–40%. Recurrence after a first relapse is 80%. The only current treatment is plasmapheresis and/or cyclophosphamide. We report successful treatment of a second relapse in a 48-year-old patient. At age 33, FSGS was diagnosed. The patient began hemodialysis 1 year later. In her first renal transplant, she developed recurrent FSGS and reached terminal transplant failure 3 years later. Eight years later, a second transplant was performed. Immunosuppressive regimen: steroids, mycophenolate mofetil (MMF), tacrolimus (TAC), and rabbit antithymocyte globulin. Proteinuria of 2–6 g/day was detected and a biopsy showed recurrent FSGS. Plasmapheresis was started without success. Another biopsy still showed FSGS. The patient received two doses of rituximab (375 mg/m^2 each) i.v. Three weeks later, proteinuria was 350 mg/day (serum-creatinine 1.6 mg/dl). Twelve months later, proteinuria was at 90 mg/day. Rituximab might be an option for recurrent FSGS after renal transplantation.

Introduction

Focal segmental glomerulosclerosis (FSGS) as a primary or secondary disease is the most frequent cause of the nephrotic syndrome in adults [1]. In its primary or idiopathic form, FSGS is found in 2–5% of patients with end-stage renal disease (ESRD) on the Eurotransplant waiting list. Because in many patients with ESRD there is no histological proof of the original disease, the exact numbers are likely higher. After transplant, FSGS is one of the most common entities leading to a recurrence of the original disease. Recurrence rates of 20–40% have been estimated [2,3]. In most cases, proteinuria recurs within 2–4 weeks after transplant. Once recurrence occurs, it leads to transplant failure within 5 years in about 10–30% of affected patients and the risk of a recurrence in subsequent grafts increases to 80% [2]. Evidence-based treatment options for recurrent FSGS do not exist. High-dose cyclosporine, TAC, cyclophosphamide, angiotensin-converting enzyme (ACE) inhibitors and steroids have been tried with varying success [2]. The most successful treatment in recurrent FSGS appears to be plasmapheresis with or without cyclophosphamide [4–6].

Rituximab is a B-lymphocyte (anti-CD20)-specific monoclonal antibody, which has been used to treat B-cell lymphoma since 1997 [7,8]. It is used increasingly in autoimmune diseases [9] and in treating antibody-mediated rejection in organ transplantation [10,11].

We here report on the use of rituximab to treat recurrent FSGS in a renal transplant patient.

Case report

The patient is a 48-year-old female who developed nephrotic syndrome at the age of 33. A kidney biopsy revealed idiopathic FSGS and the patient reached ESRD 1 year

later. After 3 years on dialysis, she received her first deceased donor kidney transplant. Her immunosuppressive regimen consisted of cyclosporine and prednisone. Three months after transplant, she developed a proteinuria of 6 g/day and a transplant biopsy showed recurrent FSGS. She was treated with high-dose steroids, but proteinuria was essentially unchanged. Three years later, the patient had to restart hemodialysis because of terminal transplant failure.

Eight years later, she received a second deceased donor kidney transplant. The patient had no panel reactive antibodies and there were no HLA mismatches. Her initial immunosuppressive regimen was: five courses of rabbit anti-thymocyte globulin, TAC (blood level 9–13 ng/ml), mycophenolate mofetil (MMF) (2 × 750 mg), and prednisone. The transplant showed immediate function and creatinine fell to 1.0 mg/dl within 8 days. Twelve days after transplant, proteinuria was detected in the routine daily dipstick control. The 24-h urinary protein was 1.4 g/day (Table 1). A first transplant biopsy showed reversible tubular damage with essentially intact foot processes in the electron microscopy and no evidence of cellular or humoral rejection (C4d in peritubular capillaries was negative). Enalapril 10 mg/day was started and the patient was released from the hospital. Forty days post-transplant, the 24-h protein excretion increased to 2–6 g. Now, the second transplant biopsy showed 10 glomeruli out of which one was segmentally and two were completely sclerosed. The remaining glomeruli displayed open capillary lumina and regular basement membranes. Occasionally, a subtle segmental mesangial matrix increase could be observed. In the interstitium, a focal edema and mononuclear cell infiltrates, partially composed of CD20 positive cells (Fig. 1a), could be seen but without any infiltration of tubular structures. Tubules showed focally accentuated epithelial cell flattening and vacuolization. Neither IgA, IgG nor complement factor C3 depositions were detectable in the glomeruli. IgM and complement factor C1q showed slight mesangial positivity. There was no nuclear SV 40 T-antigen positivity. C4d in peritubular capillaries was again negative. Electron microscopy revealed podocytes without foot processes (Fig. 1b). In summary, these changes were consistent with the diagnosis of FSGS with diffuse tubulointerstitial damage without any indication for cellular or humoral organ rejection or for viral infection.

Plasmapheresis treatment every other day was started immediately (using 5% albumin as replacement fluid). TAC dose was increased (blood level 12–20 ng/ml) while MMF (2 × 750 mg) and prednisone (10 mg/day) dosages were left unchanged. After 21 plasmapheresis sessions, proteinuria was still 6–10 g/day. Blood pressure was around 170/100 mmHg and the patient was treated with a beta-blocker, a calcium antagonist and a diuretic, enalapril was continued.

The third transplant biopsy again showed glomeruli with segmental capsular adhesions, capillary obliteration, increased mesangial matrix and foam cells, i.e. changes congruent with the diagnosis of FSGS and confirming the previous diagnosis (Fig. 1c).

Again no microscopical indications for transplant rejection, viral infection or calcineurin-inhibitor-mediated tubular toxicity were detected. C4d in peritubular capillaries remained negative. Valsartan 160 mg/day was added to the enalapril without effect on the proteinuria. Four weeks later, the patient received two weekly doses of rituximab i.v. (375 mg/m^2 each, preceded by 100-mg prednisone and 2-mg clemastine i.v.), which were tolerated well. The number of peripheral CD 19-positive B lymphocytes decreased from 213/μl before rituximab to 4/μl. Six days after the second rituximab dose, proteinuria was 2.3 g/day, and 4 weeks later it was 350 mg/day (Fig. 2) with a serum-creatinine of 1.6 mg/dl. Blood pressure decreased to 100/60 mmHg. Because of these clinical signs

Table 1. Key clinical parameters.

Days post-transplant	S-creatinine (mg/dl)	24-h urinary protein (g)	Transplant biopsy	Treatment
12	1.01	1.4	Tubular changes	Tacrolimus (TAC) (10–12 ng/ml), mycophenolate mofetil (MMF) (1.5 g/day), Pred. 25 mg/day, enalapril 10 mg/day
43	1.26	2–6	Focal segmental glomerulosclerosis (FSGS)	TAC (12–20 ng/ml), MMF (1.5 g/day), Pred. 10 mg/day, Plasmapheresis × 21
101	1.50	6–10	FSGS	TAC (10–12 ng/ml), MMF (1.5 g/day), Pred. 10 mg/day, valsartan added to enalapril
122	1.55	12		TAC (10–12 ng/ml), MMF (1.5 g/day), Pred. 10 mg/day, rituximab 600 mg i.v.
128	1.44	2.3		TAC (10–12 ng/ml), MMF (1.5 g/day), Pred. 10 mg/day rituximab 600 mg i.v.
159	1.57	0.35		TAC (10–12 ng/ml), MMF (1.5 g/day), Pred. 10 mg/day
380	1.29	0.09		TAC (7–10 ng/ml), MMF (1.0 g/day), Pred. 5 mg/day

of successful treatment, a post-treatment biopsy was not performed. Twelve months later, B-cell number remained at 3/µl, proteinuria was 90 mg/day and the serum creatinine was 1.3 mg/dl. No significant infectious complications or other discernible side effects were noted.

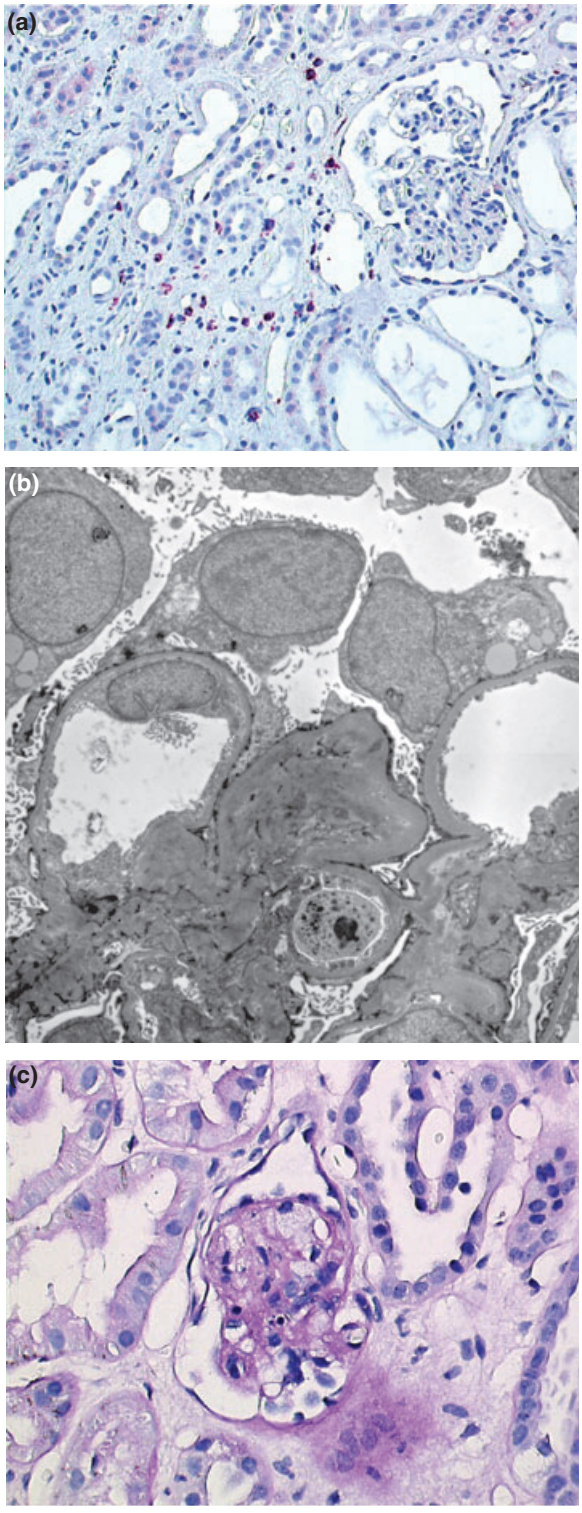

Figure 1 Light and electron microscopy studies: 3 µm paraffin-embedded sections were cut and stained with periodic acid-Schiff reagent and Goldner trichrome, respectively. Immunohistology was performed for IgA, IgG, IgM, C1q, C3, Fibrinogen/Fibrin. Immunohistochemical staining for CD20 was performed by alkaline phosphatase anti-alkaline phosphatase (APAAP) method as described in Porubsky *et al*. AJP, 2004; 164: 2175. For electron microscopy, tissue was embedded in araldite. Ultrathin sections were stained with lead citrate and viewed with a Zeiss 900 transmission electron microscope. (a) Immunohistology of CD20: Glomerulus with an adhesion of capillaries to Bowman's capsule and matrix increase. Adjacent tubulointerstitium with interspersed CD20-positive cells. Alkaline phosphatase anti-alkaline phosphatase stain. (b) Electron microscopy of a glomerulus with podocytes without foot-processes and with lipid droplets in cytoplasm and microvilli-like protrusions; mesangium shows an increase in matrix; 3000×. (c) Glomerulus showing segmental sclerosis with occlusion of the tubular pole. Several foam cells can be seen in an increased matrix. PAS stain.

Discussion

This is, to the best of our knowledge, the first report describing the successful use of rituximab intended as a treatment of recurrent FSGS in a renal allograft. Recurrence of FSGS after renal transplantation is estimated to occur in about 20% of patients with primary FSGS as the cause of ESRD [2,3]. In patients younger than 20 years with a rapid course of their original disease, the recurrence rate may reach 50% [12,13]. The pathogenesis of the original disease and its recurrence have not been elucidated yet [1]. A circulating nonimmunoglobulin factor of 30–50 kDa has been implicated [4,14,15]. But the exact nature of this protein remains elusive [1]. This concept has been fostered by studies showing successful treatment of FSGS recurrence by plasmapheresis or protein absorption techniques [4,5,12,16,17] and by the finding that patients with familial FSGS because of a NPHS2 mutation may also develop recurrent FSGS after renal transplantation [18].

Plasmapheresis or protein absorption has therefore been used in many transplant centers to treat relapsed FSGS after transplantation. Long-term remissions, however, are achieved in only 40–50% of patients, some patients appear to be plasmapheresis-dependent with immediate recurrence of nephrotic proteinuria once plasmapheresis is stopped [5,17]. In studies in children, the success rate could apparently be increased by adding cyclophosphamide to the plasmapheresis [6,19]. Cyclosporine can be used with some success in the primary disease and has therefore been tried in high doses to treat recurrence [20] (despite the fact that in the last 20 years most recurrences developed during cyclosporine therapy).

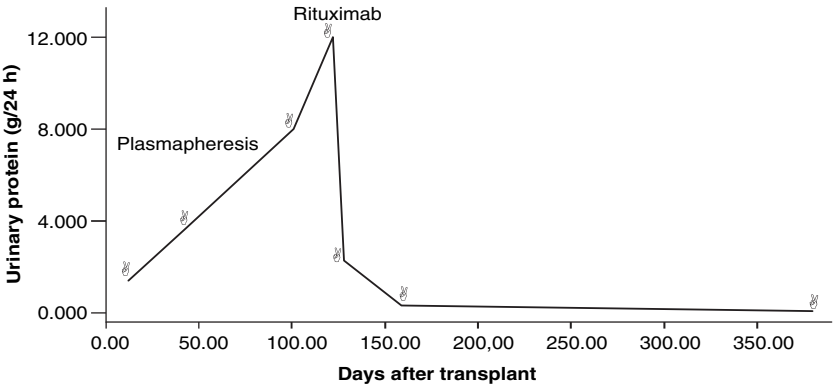

Figure 2 Twenty-four hour urinary protein excretion at different time points after transplantation and the therapeutic interventions.

In children, this approach was apparently successful in about 80% [21,22]. But in adults, treatment with a calcineurin inhibitor (CNI) in such patients usually results in nephrotoxicity and not in remission of the nephrotic syndrome.

In our patient, the risk of relapse of the FSGS was increased because of the recurrence in the previous graft. Furthermore, prolonged treatment with plasmapheresis was unsuccessful. We therefore had to search for an alternative treatment and decided to use rituximab. This decision was based on our positive experience with regard to side effects in using the antibody in patients with vascular rejection and the good safety record of rituximab [8]. The patient was fully informed about the experimental nature of the treatment. We also found a report on regression of nephrotic syndrome in a 16-year-old boy who had primary FSGS and developed immune thrombocytopenic purpura (ITP). He received rituximab as a treatment of the latter disease and had a remission of both the ITP and the nephrotic syndrome. [23]. Meanwhile, two reports on the influence of rituximab treatment on recurrent FSGS in pediatric renal transplant patients treated for post-transplant lymphoproliferative disease (PTLD) appeared [24,25]. Both showed a drastic decrease of proteinuria after successful treatment of the PTLD with rituximab. In these latter reports, rituximab was used to treat a neoplastic disease and one might therefore speculate that the FSGS could have been a paraneoplastic disease which recurred after the tumor was treated successfully. More recently, Hristea *et al.* [26] reported on a 22-year-old patient who developed rFSGS despite pre- and postoperative plasmapheresis. He received two doses of rituximab and, for 18 days, oral cyclophosphamide with additional plasmapheresis sessions and enjoyed full remission of the nephrotic syndrome for more than 2 years.

Our case is unique in that the patient had no concomitant disease; she was treated with rituximab after cessation of plasmapheresis and the sole aim of treatment was the FSGS recurrence.

Concerning the pathogenetic basis of the successful treatment of vascular rejection, autoimmune diseases and now possibly FSGS recurrence by rituximab, we can only speculate. As the plasma levels of IgG, IgM and also of specific antibodies are preserved despite treatment for prolonged periods and because the plasma cells are spared from the effects of rituximab [27,28], the elimination of pathogenic antibodies does not appear to be involved. Because B lymphocytes also have important roles as immunoregulatory cells both by antigen presentation and the secretion of cytokines, we believe that their elimination has dampening effects on other immune cells like T lymphocytes, dendritic cells, or macrophages. This might also explain the finding that the elimination of plasma proteins by plasmapheresis had no effect in this case while the elimination of the B cells had. The fact that CD20-positive B cells could also be identified in our transplant biopsy may also be a hint to their active participation in the immunological process by secreting – or inducing other cells to secrete – the putative FSGS factor [1]. A recent study in nontransplant patients with membranous nephropathy, minimal change disease, FSGS, and kidneys from tumor nephrectomies revealed significant interstitial CD20 mRNA expression only in the patients with membranous nephropathy [29]. In transplanted kidneys, CD20-positive cells have mostly been found during rejection, portending a poor prognosis [30]. But conclusive information about the role of B lymphocytes in either recurrent disease or chronic rejection or tolerance in transplanted kidneys is so far missing.

Taking our own data and the other case reports together, a prospective-randomized trial of rituximab treatment in recurrent FSGS including research into the role of the B lymphocytes appears clearly warranted.

This case report has been presented at the ASN Meeting in Saint Louis in November 2005.

Authors' contribution

J.G., E.-H.S., H.-G.K, H.G. and I.H. were all involved in the treatment of the patient, data collection and analysis and contributed to the writing of the paper. S.P. provided the histopathology and contributed to the writing of the paper.

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