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ORIGINAL ARTICLE

Elisabeth Dittrich
Sabine Schmaldienst
Afschin Soleiman
Walter H. Hörl
Erich Pohanka

Rapamycin-associated post-transplantation glomerulonephritis and its remission after reintroduction of calcineurin-inhibitor therapy

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E. Dittrich · S. Schmaldienst (✉)
W. H. Hörl · E. Pohanka
Department of Medicine III,
Division of Nephrology and Dialysis,
University of Vienna, 1090 Vienna,
Austria
E-mail:
sabine.schmaldienst@akh-wien.ac.at
Tel.: +43-1-404004390
Fax: +43-1-404004392

A. Soleiman
Department of Pathology,
University of Vienna, Vienna,
Austria

Abstract Rapamycin is a new immunosuppressive agent approved for maintenance therapy after kidney transplantation. It may allow calcineurin-inhibitor-free, non-nephrotoxic immunosuppression. We report, however, on four kidney-transplant recipients who developed post-transplantation glomerulonephritis after conversion from a calcineurin-inhibitor-based immunosuppression to rapamycin. In all four patients nephrotic-range proteinuria occurred 2–9 months after conversion to rapamycin. Renal biopsy confirmed membrano-proliferative glomerulonephritis type 1 in one case, membranous glomerulonephritis in another and IgA-nephropathy in two cases, respectively. Calcineurin-inhibitor-based immunosuppression was reintroduced and resulted in complete remission of proteinuria and in stabilised renal function in all patients. We conclude that in the case of rapamycin-associated post-transplantation glomerulonephritis an attempt should be made to replace rapamycin by a calcineurin inhibitor.

Keywords Calcineurin inhibitor · Glomerulonephritis · Rapamycin · Renal transplantation

Introduction

Rapamycin is a new, promising immunosuppressive drug introduced for maintenance therapy after solid-organ transplantation [1, 2]. Because of its power to suppress acute rejections, the substance is currently under investigation in calcineurin-inhibitor-sparing regimens after kidney transplantation. Replacement of the nephrotoxic agents by rapamycin might reduce the risk for chronic allograft dysfunction and could thereby lead to an overall improvement of kidney graft survival [3, 4]. However, many physicians express their concerns that the withdrawal of calcineurin inhibitors, which have substantially contributed to the success of organ transplantation in general, might put their patients at risk of insufficient immunosuppression.

Post-transplantation glomerulonephritis is a well-known feature that may develop "de novo" or as recurrence of the original disease in a patient's graft, and it can be observed in all immunosuppressive drug combinations. What makes the four cases, reported here, quite unique, is the occurrence of proteinuria and biopsy-proven glomerulonephritis during rapamycin therapy after the withdrawal of calcineurin inhibitors, and the remission after the withdrawal of rapamycin and reintroduction of calcineurin-inhibitor therapy.

Patients and treatment modalities

The four patients reported here underwent kidney transplantation at the University of Vienna Medical

Faculty (general hospital) between 1995 and 2001. Maintenance immunosuppressive therapy after transplantation was based on steroids, calcineurin inhibitors and proliferation inhibitors (mycophenolate mofetil in three patients, azathioprine in one patient). Between November 2000 and March 2003 the four patients were switched from the calcineurin-inhibitor-based immunosuppressive treatment to a rapamycin-based therapy regimen. The patients' treatments were changed 11 months–48 months after transplantation: in three patients because of impaired or slowly deteriorating graft function, and in one patient because of gingival hyperplasia.

Patient 1

A 57-year-old male Caucasian was started on haemodialysis in 1995 because of end-stage renal failure of unknown origin and lost his first renal transplant due to infectious complications within 4 weeks of transplantation. He received a second allograft in October 1999. During post-transplantation hospitalisation he was treated for one acute rejection (Banff grade 1) with steroid-pulse therapy and was discharged with stable but moderately impaired renal function. During the first post-transplantation year serum creatinine (sCr) ranged from 2.2–2.5 mg/dl (194–221 μmol/l). Maintenance immunosuppression consisted of cyclosporine (trough levels 150–200 ng/ml), mycophenolate mofetil (2 g/day) and low-dose prednisolone (5 mg/day). Fourteen months after receiving the graft the patient participated in a clinical trial to test the impact of cyclosporine withdrawal in patients with chronic allograft dysfunction. A renal biopsy was performed to rule out acute rejection or any glomerular disease (Table 1) before the patient's treatment was converted to rapamycin and steroids (termination of cyclosporine and mycophenolate mofetil) (Fig. 1A). Rapamycin target range was 12–20 ng/ml (determined by HPLC), and prednisolone was transiently increased to 15 mg/day and consequently tapered thereafter to 5 mg/day.

After the patient had undergone 9 months of rapamycin therapy, sCr had increased from 2.5 mg/dl to 4.0 mg/dl (221–354 μmol/l), and proteinuria of 2.5 g/24 h had developed. A renal biopsy showed acute rejection (Banff borderline) and, in addition, membranoproliferative glomerulonephritis type 1 (Fig. 2, Table 1). No signs of transplant glomerulopathy could be found under light microscopy and electron microscopy. After steroid-pulse therapy for rejection the patient's treatment was converted to the initial cyclosporine-based immunosuppression. During the next weeks sCr decreased to 3.0 mg/dl (265 μmol/l) and remained stable thereafter. Urinary protein excretion dropped and was no longer detectable 4 months later (Fig. 1A). Another renal biopsy was performed 7 months after termination of rapamycin treatment and reintroduction of cyclosporine. The glomerulonephritis-associated lesions had disappeared, confirming the complete remission of the glomerular disease (Table 1). One year after conversion sCr was 3.1 mg/dl (274 μmol/l).

Patient 2

A 57-year-old male Caucasian who suffered from end-stage renal failure due to adult polycystic kidney disease was started on haemodialysis in 1992. He lost his first kidney transplant due to repeated acute rejections within 5 months. The second renal transplant was lost due to membranous glomerulonephritis with severe nephrotic syndrome refractory to any anti-proteinuric therapy and even immunoadsorption. The third renal

Table 1 Histology of repeated biopsies (*GN* glomerulonephritis)

Patient no.	Biopsy before switch to rapamycin	Biopsy under rapamycin therapy	Biopsy after termination of rapamycin therapy
1	Regular parenchyma. No signs of acute rejection, glomerulonephritis or transplant glomerulopathy	**Membrano-proliferative GN type I** (immune-complex deposits); acute rejection Banff borderline; low-grade chronic allograft nephropathy	No signs of acute glomerulonephritis (no immune-complex deposits detectable); low-grade chronic allograft nephropathy
2	Low-grade chronic allograft nephropathy. No signs of acute rejection, glomerulonephritis or transplant glomerulopathy	**Membranous glomerulonephritis**; low-grade chronic allograft nephropathy	
3		**IgA nephropathy** (mesangio-proliferative); medium-grade chronic allograft nephropathy	
4		**IgA nephropathy** (segmental sclerosis—1/6 glomeruli); low-grade chronic allograft nephropathy	IgA nephropathy (segmental sclerosis—1/12 glomeruli)

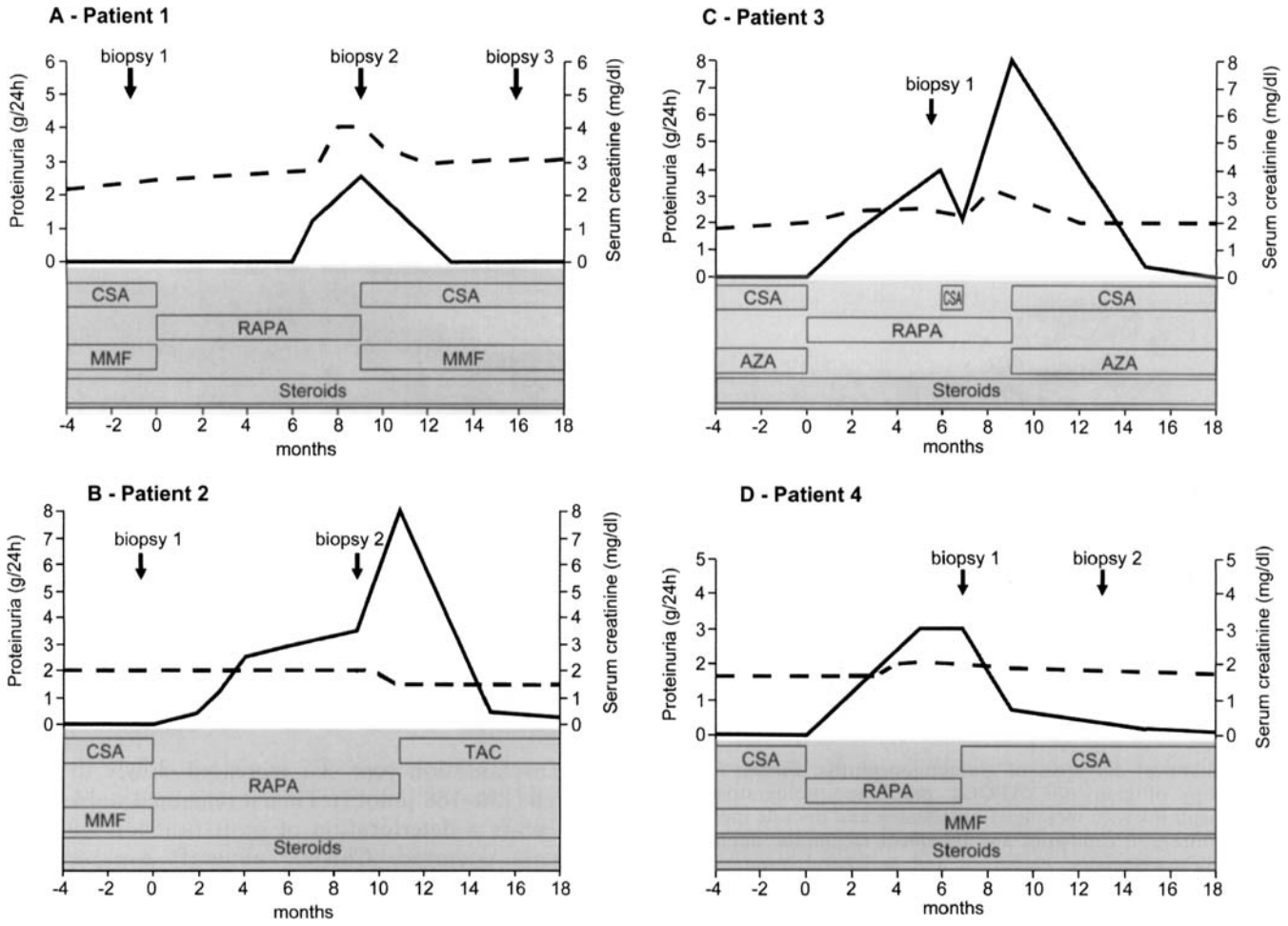

Fig. 1 Course of proteinuria and serum creatinine. Month 0 is the time of introduction of rapamycin. *Black arrows* indicate biopsies. *CSA* cyclosporine, *MMF* mycophenolate mofetil, *RAPA* rapamycin, *AZA* azathioprine, *TAC* tacrolimus, *solid lines* proteinuria levels, *dotted lines* serum creatinine

allograft was transplanted in March 2001. Because of the patient's sensitivity to HLA (cytotoxic antibodies 100%) the initial immunosuppressive regimen was a combination of cyclosporine, mycophenolate mofetil, steroids, antithymocyte globulin and immunoadsorption. No acute rejection occurred during the post-transplantation follow-up. The patient was discharged from hospital with a stable sCr of 2.0 mg/dl (177 μmol/l). Triple-drug maintenance immunosuppression consisted of cyclosporine (trough-levels 150–200 ng/ml), mycophenolate mofetil (2 g/day) and prednisolone (tapered to 5 mg/day). Fourteen months after receiving the graft he participated in the clinical trial for cyclosporine-withdrawal in patients with chronic allograft dysfunction (see patient 1). The renal biopsy, performed prior to conversion, showed low-grade chronic allograft nephropathy but no signs of acute rejection, transplant glomerulopathy or glomerulonephritis (Table 1). The patient's immunosuppressive regimen was then switched to rapamycin/prednisolone according to the protocol described for patient 1 (Fig. 1B).

Nine months after conversion to rapamycin the patient developed proteinuria (3.5 g/24 h), but sCr remained stable at 2.0 mg/dl (177 μmol/l). A renal biopsy showed membranous glomerulonephritis (verified by electron microscopy) and low-grade chronic allograft nephropathy (Fig. 2). Within the next 2 months there was worsening of proteinuria to 8 g/24 h, despite increased ACE inhibitor dosage and newly introduced angiotensin II receptor-antagonist therapy. There was clinical evidence of nephrotic syndrome with massive oedema and weight gain. At this point immunosuppression was switched back to a calcineurin-inhibitor-based regimen [now tacrolimus (trough level 10–15 ng/ml) and prednisolone (10 mg/day for 4 weeks and then 5 mg/day for maintenance therapy)]. Proteinuria decreased thereafter and was 0.4 g/24 h 4 months after introduction of tacrolimus (Fig. 1B). Renal function improved to a sCr of 1.5 mg/dl (133 μmol/l).

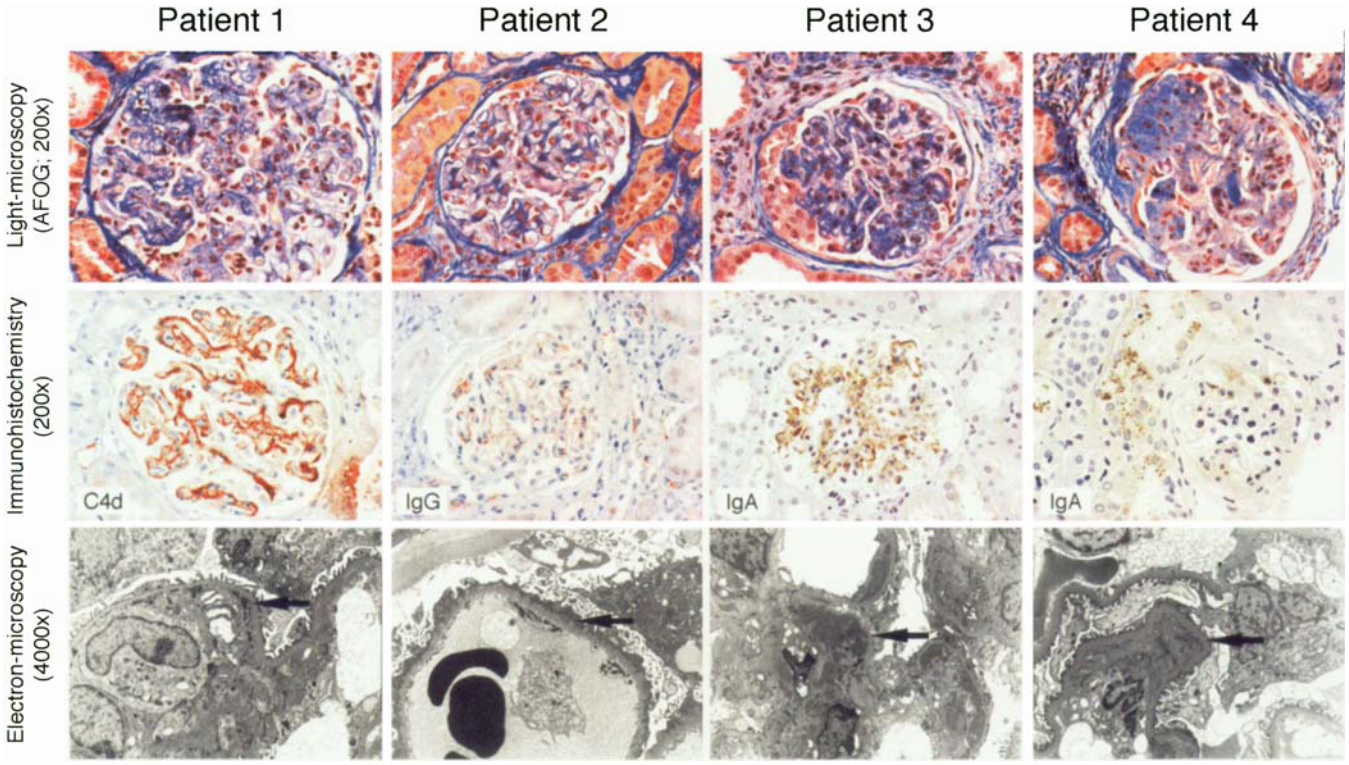

Fig. 2 Bioptical diagnosis of glomerulonephritis. *Patient 1* light microscopy of glomeruli (AFOG): membrano-proliferative glomerulonephritis with mesangial broadening and discrete mononuclear infiltrate in capillaries and basement membrane duplicates. Immunohistochemistry: mesangial and peripheral immune-complex deposits with strong staining of complement components (C4d). Electron microscopy: electron-dense immune-complex deposits in the mesangium (*arrow*). *Patient 2* light microscopy of glomeruli (AFOG): discrete mesangial broadening and thickening of peripheral basement membranes. Immunohistochemistry: granular mesangial and sub-epithelial immune-complex deposits characteristic for membranous glomerulonephritis (IgG). Electron microscopy: granular sub-epithelial immune-complex deposits with basement membrane spikes (*arrow*). *Patient 3* light microscopy of glomeruli (AFOG): IgA nephropathy with mesangial proliferation, segmental sclerosis and crescent formation. Immunohistochemistry: numerous mesangial and sub-endothelial immune-complex deposits positive for IgA. Electron microscopy: electron-dense immune-complex deposits in the mesangium (*arrow*) and in sub-endothelial localisation. *Patient 4* light microscopy of glomeruli (AFOG): IgA-nephropathy with severe mesangial and segmental sclerosis but minimal proliferation. Immunohistochemistry: discrete mesangial deposits of IgA. Electron microscopy: electron-dense immune-complex deposits in the mesangium (*arrow*)

Patient 3

This patient was a 42-year-old Caucasian woman with mesangio-proliferative glomerulonephritis in her native kidneys. In 1995 she underwent living-related donor kidney transplantation, with excellent primary graft function. Immunosuppressive therapy consisted of cyclosporine, azathioprine and steroids. During the first post-transplantation year sCr increased slowly to 1.7–1.9 mg/dl (150–168 μmol/l). Then it remained stable, but after 4 years a deterioration of graft function without proteinuria occurred. Chronic allograft nephropathy and/or cyclosporine-associated nephrotoxicity were considered clinically, and maintenance immunosuppression was replaced by combination of rapamycin (target level 10–15 ng/ml) and prednisolone (dose unchanged, 2.5 mg/day).

Two months later microhaematuria and proteinuria of 1.6 g/24 h developed. Urinary protein excretion increased to 4 g/24 h at 6 months after conversion (Fig. 1C). A renal allograft biopsy was performed at a sCr of 2.5 mg/dl (221 μmol/l), which revealed IgA-mesangio-proliferative glomerulonephritis and medium-grade chronic allograft nephropathy (Fig. 2, Table 1). Losartan (50 mg/day) was added to a constant therapy with ramipril (5 mg/day), and cyclosporine was added to rapamycin/steroids for 4 weeks. Within this time proteinuria dropped to 2.1 g/24 h. Cyclosporine therapy was then interrupted for 5 weeks, and urinary protein excretion increased again to 8 g/24 h (Fig. 1C). SCr reached 3.2 mg/dl (283 μmol/l), but the patient refused a second biopsy. She received steroid-pulse therapy, and, finally, rapamycin was replaced by the original drug combination (cyclosporine, azathioprine and steroids). Proteinuria decreased to 0.25 g/24 h 5 months later and was not detectable after 10 months. SCr improved to a stable level of 2.0 mg/dl (177 μmol/l) during a follow-up of 21 months.

Patient 4

Patient 4 was a 35-year-old male Caucasian who underwent pre-emptive renal transplantation (living-related donor) in September 2001 because of end-stage renal failure due to biopsy-proven glomerulopathy with segmental sclerosis. Post-transplantation sCr was 1.8 mg/dl (159 μmol/l), and it remained stable thereafter. Maintenance triple-drug immunosuppressive therapy consisted of cyclosporine (trough-level 150–200 ng/ml), mycophenolate mofetil (2 g/day) and prednisolone (5 mg/day). Because the patient suffered massive gingival hyperplasia, 11 months after he had received the graft cyclosporine was replaced by rapamycin, with ongoing mycophenolate mofetil in a lower dose (1.5 g/day, further tapered to 1 g/day because of gastrointestinal symptoms) and prednisolone (5 mg/day). No proteinuria was detectable at time of conversion. During the following 5 months the patient developed proteinuria up to 3 g/24 h (Fig. 1D), and urinary protein excretion remained at this level despite introduction of an ACE inhibitor and angiotensin II receptor-antagonist therapy.

After the patient had suffered another 2 months of consistent proteinuria, a renal biopsy was performed, which showed IgA-nephropathy with segmental sclerosis (1/6 glomeruli) and low-grade chronic allograft nephropathy (Fig. 2, Table 1). Immunosuppression was converted to the previous calcineurin-inhibitor-based regimen [cyclosporin (trough-level ~120 ng/ml), mycophenolate mofetil (1.5 g/day) and prednisolone (5 mg/day)]. Proteinuria decreased to 0.5 g/day. Six months later a further biopsy showed chronic lesions with segmental sclerosis in 1/12 glomeruli; staining for IgA was slightly positive in immunohistochemistry. Twelve months after reintroduction of cyclosporine the proteinuria was 0.25 g/24 h with a stable sCr of 1.8 mg/dl (159 μmol/l).

Discussion

Post-transplantation glomerulonephritis, which may occur "de novo" or as recurrence of disease, is a well-known complication after renal grafting with a possible negative impact on long-term graft survival. The glomerular lesions develop despite ongoing immunosuppression and are observed in all drug combinations. Although therapeutic benefits have been suggested for mycophenolate mofetil [5, 6], cyclosporine [7] and tacrolimus [8] in several case reports or smaller series, no successful treatment for post-transplantation glomerulonephritis has been established in general [9].

In this paper we report the course of four patients with several similarities. Prior to intervention all patients had chronic allograft dysfunction but had no evidence of glomerular disease, which was even ruled out by kidney biopsy in two cases. All patients were switched to rapamycin treatment to test calcineurin-inhibitor-free immunosuppression, because of concerns about possible calcineurin-inhibitor-induced nephrotoxicity in three cases or gingival hyperplasia in one case. Following conversion to rapamycin treatment all patients developed proteinuria, resulting in transplant biopsy after several months. None of the four patients had viral infection, malignancy or had received antithymocyte globulin therapy within the 12 months prior to the occurrence of proteinuria, which might have triggered post-transplantation glomerulonephritis. In all patients proteinuria resolved completely after reintroduction of calcineurin-inhibitor-based immunosuppression, although calcineurin inhibitors do not prevent post-transplantation glomerular lesions in general. The additionally administered steroid-pulses in two cases might have contributed to the remission. However, complete remission occurred not only in those patients with steroid pulse therapy. Although the clinical course of post-transplantation glomerulonephritis can be benign, spontaneous remissions are rare, particularly in the case of membrano-proliferative glomerulonephritis and IgA-nephropathy.

It can be speculated that rapamycin was unable to suppress immune activation and the glomerular injury sufficiently, or that the drug itself might have actively triggered such a process. Usually, the replacement of a calcineurin inhibitor by rapamycin is a safe option and can lead to improvement of renal graft function [4, 10]. At present there are no studies indicating that rapamycin would induce post-transplantation glomerulonephritis in a clinical setting, but quite recently, new onset and worsening of proteinuria after introduction of rapamycin has been reported in solid-organ transplant recipients [11, 12]. After discontinuation of rapamycin, proteinuria declined in accordance with our observation. In an experimental setting rapamycin was able to trigger an inflammatory process in the glomeruli. Through the use of a rat model the rapamycin derivative SDZ-RAD has been shown to accentuate glomerular damage by marked pro-inflammatory effects, thereby aggravating the course of immune-complex glomerulonephritis [13].

The course of patient 3, however, seems to indicate that the reintroduction of the calcineurin inhibitor was critical for achieving remission. In this case proteinuria decreased immediately after a short administration of cyclosporine, which was then administered in addition to rapamycin (Fig. 1C). This favours the hypothesis that the calcineurin inhibitor was more effective in suppressing the inflammatory response in the graft. Withdrawal of the calcineurin inhibitor might then have allowed immune activation (proteinuria increased to 8 g/24 h after termination of cyclosporine), which was finally terminated by reintroduction of the calcineurin inhibitor.

Mycophenolate mofetil has been effective in the treatment of nephrotic syndrome [14], various autoimmune diseases [15] and in post-transplantation glomerulonephritis [5, 6]. Therefore, a possible role for proliferation inhibitors could be suspected. However, mycophenolate mofetil or azathioprine were withdrawn and reintroduced in just two patients (Fig. 1A, C). Thus, those two drugs can hardly account for the occurrence and the remission of proteinuria observed in all patients.

All patients were treated with ACE inhibitors; three patients were even treated in combination with an angiotensin II receptor antagonist. Although both substances have certain haemodynamic effects and, thus, can reduce proteinuria, complete remission of nephrotic-range proteinuria cannot be expected. Besides, the histological features of glomerulonephritis have been resolved (demonstrated by repeated biopsies), which cannot be explained by haemodynamic changes. Furthermore, the time course of increasing and then decreasing proteinuria did not correlate with the use or the dosage of these drugs.

At present, an increasing number of graft recipients are treated with rapamycin-based immunosuppression after withdrawal of calcineurin inhibitors. Our experience indicates that careful monitoring of urinary protein excretion is recommended in these patients and that their re-conversion to calcineurin inhibitors can be a possible option for treatment of post-transplantation glomerulonephritis. In general, we believe that any changes in immunosuppression should be handled with extreme caution.

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