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ORIGINAL ARTICLE

Treatment of symptomatic transplant glomerulopathy with rituximab

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Summary

Kidney transplant glomerulopathy (TG) has a poor outcome as there are no known effective therapies. Therefore, we investigated whether rituximab therapy (RTx) could halt progression of established TG. Fourteen kidney-transplant patients (nine of whom were men), with median age of 54 (range: 30–74) years, of whom seven had biologic markers for HCV infection, underwent a kidney biopsy (KB) at 118 months post-transplant because of impaired allograft function, associated with albuminuria (95–13430 mg/day), within nephrotic-range albuminuria in seven patients. KBs showed no evidence of acute cellular rejection but showed TG. Donor-specific anti-HLA antibodies were present in six cases. When diagnosis of TG was made, patients were placed on rituximab therapy (RTx) (2–4 injections of 375 mg/m^2 week), and other concurrent immunosuppression treatments were not modified. By last follow up post-RTx (30 months), seven (50%) patients had lost their kidney within 6–26 months and the other seven had stable creatinine (182 vs. 161 μmol/l; NS), and albuminuria had decreased from 2660 to 500 mg/day ($P = 0.03$). There was prolonged B-cell lymphopenia (from 71 to 0/mm^3) whereas immunoglobulin G, A, M levels remained stable, and four patients (28.5%) experienced severe infectious complications. We conclude that long-term RTx in kidney-transplant patients with TG is associated with allograft function/stabilization in 50% of cases.

Introduction

Transplant glomerulopathy (TG) is a distinctive glomerular lesion that is unique to kidney allografts. It is characterized, by light microscopy, as capillary-wall widening and double contours that enclose a clear zone that is often contiguous with a pale expanded mesangium. Electron microscopy shows that the thick double glomerular capillary outlines are caused by flocculent material. This is distinguished from mesangiocapillary glomerulonephritis by the absence of mesangial proliferation and lack of immune deposits as shown by immunofluorescence (or nonspecific mesangial IgM and C3) and electron microscopy [1–3]. TG features evolve over time, i.e., the earliest change being diffuse swelling of endothelial and mesangial cells with diminished patency of the capillary loops, progressing to double contours of glomerular basement membranes (GBMs) and an increase in mesangial matrix, sometimes with segmental sclerosis [4]. TG is relatively rare when compared with other lesions encountered in surveillance biopsies obtained 1 year after transplantation in patients with well-functioning allografts, often with minimal or no other histologic abnormalities [5]. However, although this early presentation may be associated

with relatively mild degrees of allograft dysfunction at diagnosis, the outcome of TG is poor [6].

To determine the etiologic factors for TG, Gloor *et al.* [7] studied a series of 582 patients of whom 55 had TG. Acute rejection, hepatitis C antibody positivity, and anti-HLA antibodies, especially anti-class II antibodies were associated with the development of TG. The risk of TG increased if anti-HLA antibodies were donor-specific (DSA). TG has also been associated with C4d deposits in peritubular capillaries [3,8,9]. The Edmonton group identified variable forms of TG according to the presence of four components (the so-called ABCD tetrad): antidonor antibodies (A), peritubular capillary basement-membrane multilayering on electron microscopy examination (B), peritubular capillary C4d deposition (C), and GBM duplication (D) [3]. However, recently, Akalin *et al.*, [1] in a cohort of 36 patients with TG, found that 89% and 64% of patients were C4d- and DSA-negative respectively. A link between shortened allograft survival and anti-HLA class II antibodies is that HLA class II antigens are strongly expressed on endothelial cells of the peritubular and glomerular capillaries; this distribution corresponds to the areas in which C4d deposition is noted in both acute as well as chronic antibody-mediated rejection (AMR) [10].

The fact that low-level anti-HLA class II antibody activity is strongly associated with the chronic glomerular and peritubular vasculopathy of TG, and that anti-HLA class II antigen expression corresponds to this same capillary bed provides further evidence that TG is a manifestation of chronic AMR [2]. Currently, there are no known effective therapies for TG, apart from controlling blood pressure and using angiotensin II inhibition: these slow the progression of glomerular diseases on native kidneys [2]. Rituximab has been used for treating several glomerulopathies after kidney-transplantation [11–17]. Because of TG pathogenesis, we performed a preliminary study to evaluate the safety profile of rituximab in patients with established TG.

Patients and methods

We included 14 kidney-transplant patients, of whom 12 were recipients of a deceased donor's allograft. This was their first transplant in six cases, and an iterative one in the remainder. There were nine male patients and five female patients of median age 54 (30–74) years. Reasons for kidney transplantation were chronic glomerulopathy in 13 cases and interstitial nephritis in one patient (Table 1). Seven of the 14 (50%) patients had biologic markers for hepatitis C virus infection, i.e., they tested positive for both anti-HCV antibodies and HCV RNA. This study was approved by our Institutional Review Board, and all patients gave their informed consent.

Maintenance immunosuppression was the following: all patients but three received mycophenolate mofetil (MMF) at 1000 (1000–2000) mg/day; two other patients received azathioprine (AZA) 50 mg/day, whereas the last did not receive either MMF or AZA. One patient was on sirolimus-based therapy (1.5 mg/day), whereas the others were on calcineurin inhibitors, i.e., cyclosporine in five cases aiming at C2 of between 400 and 800 ng/ml and tacrolimus in six cases aiming at trough levels of between 5 and 10 ng/ml. Thus, two patients did not receive cyclosporine/tacrolimus or sirolimus-based therapies. All but three patients were on low-dose prednisone [5 (2–10 mg/day)] (Table 1). Since transplantation, only four patients have

Table 1. Patients' characteristics before rituximab therapy.

	Cause of ESKD	Age (years)	HCV (Y/N)	KT to RTx (mo)	Time since RTx (mo)	IS at RTx
Patient 1	Interstitial nephritis	65	N	170	18	C/MMF/Cs
Patient 2	HUS	63	N	84	24	T/MMF/Cs
Patient 3	MPGN	42	Y	20	26	T/MMF/Cs
Patient 4	IgA GN	47	Y	184	22	C/MMF/Cs
Patient 5	CGN	53	Y	255	19	MMF/Cs
Patient 6	IgA GN	69	N	165	24	C/MMF/Cs
Patient 7	CGN	54	Y	51	26	T/MMF/Cs
Patient 8	CGN	33	Y	104	31	C/MMF/Cs
Patient 9	MPGN	55	Y	121	42	T/MMF/Cs
Patient 10	CGN	40	N	121	21	C/Cs
Patient 11	MPGN	59	Y	239	25	MMF/Cs
Patient 12	Alport	30	N	74	16	T/MMF/Cs
Patient 13	IgA GN	36	N	108	14	C/MMF/Cs
Patient 14	MPGN	74	N	118	12	S/MMF/Cs

HUS, hemolytic and uremic syndrome; MPGN, membranoproliferative glomerulonephritis; IgA GN, glomerulonephritis with mesangial IgA deposits; CGN, chronic glomerulonephritis; HCV, hepatitis C virus; KT, kidney transplantation; RTx, rituximab therapy; Y, yes; N, no; IS, immunosuppression; C, cyclosporine; T, tacrolimus; S, sirolimus; MMF, mycophenolate mofetil; Cs, steroids.

had episodes of acute cellular rejection, which were successfully treated by methylprednisolone pulses ($n = 2$) or by OKT3 ($n = 2$).

Patients underwent a KB at 112 (20–255) months post-transplant because they had developed impaired allograft function, i.e., serum creatinine had increased over the 3-month period before the KB from 146 (122–254) to 161 (130–308) μmol/l ($P < 0.01$), taking into account the decrease in calculated creatinine clearance (CC; Cockcroft and Gault formula) from 45 (30.7–74.7) to 39 (25.4–57) ml/min ($P < 0.01$), associated with albuminuria of 2350 (95–13430) mg/day. At the time of the KB, all patients were already on maximal doses of angiotensin-converting enzyme inhibitors (ACEIs, $n = 3$) or angiotensin II receptor blockers (ARBs, $n = 9$), or both ($n = 2$) therapies. Moreover, all patients had hypertension, which was well-controlled with various antihypertensive drugs.

The patients' biologic workup at the time of the KBs included antinuclear (ANA) and antineutrophil cytoplasmic (ANCA) autoantibodies, serum immunoelectrophoresis, total complement (CH50) as well as its subfractions C3 and C4, cryoglobulinemia, and assessment of DSAs. When the diagnosis of transplant glomerulopathy was made, and after ruling out the recurrence of the original kidney disease, the patient was placed on rituximab therapy The following parameters were recorded before each rituximab injection, then monthly for 3 months, and then at 3-monthly intervals: vital signs, including body temperature, weight, blood pressure, serum creatinine, calculated CC, dipsticks, hemoglobin; white blood-cell, polymorphonuclear, and platelet counts, and aspartate and alanine aminotransferase levels. Also, 24-h albuminuria, and lymphocyte subsets, i.e., CD3, CD4, CD8, CD19 counts, were assessed before rituximab therapy, at 15 days, 1 month, and then at 3-monthly intervals. Serum immunoglobulin levels (Ig G, Ig M, and IgA), as well as cryoglobulinemia levels, were assessed before rituximab therapy and at 1, 3, 6 months, then at 6-monthly intervals.

Cryoglobulinemia was assessed as previously reported by Faguer *et al.* [18]. DSAs were looked for in real time with ELISA techniques, and were re-assessed retrospectively in June 2008 using the Luminex technique.

Kidney biopsies were examined (optically and by immunofluorescence techniques) by a single pathologist (CGF) and were classified according to the Banff 07 criteria [19]. No ultrastructural study by electron microscopy was performed.

Rituximab therapy

Rituximab therapy dosage was chosen as follows: the three first patients were given arbitrarily two doses of rituximab (375 mg/m^2) within an interval of 7 days. Because 2 weeks after the two rituximab infusions, some peripheral B cells were still detectable, we then decided to give for the next three patients three doses of rituximab (375 mg/m^2 per week). When we evaluated rituximab therapy in the first six patients because four of the first six patients had a dramatic improvement regarding albuminuria, we injected rituximab weekly up to four doses in the remaining patients. Thus, rituximab therapy was given weekly for 2 consecutive weeks in three cases, for 3 consecutive weeks in three cases, and for 4 consecutive weeks in eight cases. Each infusion was preceded by a methylprednisolone injection of 1 mg/kg. Maintenance immunosuppression was not modified when rituximab therapy was implemented. No systematic prophylaxis was given regarding cytomegalovirus or *Pneumocystis jirovecii*.

Statistical analyses

Reported values represent either mean ± SE or medians (ranges). Proportions were compared by the chi-squared test or Fisher's exact test. Quantitative variables were compared by the nonparametric Mann–Whitney test. During treatment follow up, quantitative variables were compared by the nonparametric Friedman test for serial measurements, and the Wilcoxon test. A P-value <0.05 was considered statistically significant.

Results

Kidney biopsies

The KBs showed no evidence of acute cellular rejection. No patient had evidence of recurrence of original glomerulopathy, except the one identified as patient 10 who presented with a recurrence of immunotactoid glomerulopathy. All the biopsies showed transplant glomerulopathy, i.e., cg1 in five cases, cg2 in two cases, and cg3 in seven cases (Table 2). Aspects of membranoproliferative glomerulonephritis (MPGN) were present in six cases, whereas aspects of interstitial fibrosis and tubular atrophy were present in eight cases. Of those patients who were HCV RNA positive, only one (patient 7) had a KB showing an aspect of MPGN: he also had anti-HLA class II DSA. No patient had features of cryoglobulinemic glomerulonephritis. Dilation of peritubular capillaries was present in 13 out of 14 cases, capillaritis in two cases, and C4d was negative in all cases but one (focal). Immunostainings for the presence of T and B lymphocytes was performed on kidney biopsies before instituting rituximab therapy in 10 patients. Amongst the lymphocytes, T cells accounted for 50% (0–95), whereas B cells accounted for 25% (0–60). After rituximab therapy, a kidney biopsy was repeated in four cases: it always found the absence of B cells. There was no correlation between the percentage of

Table 2. Chronic Banff scores for each patient's kidney biopsy.

	DSA	C4 d (Y/N)	Cg	ci	ct	cv	mm
Patient 1	N	N	1	1	1	1	1
Patient 2	Y	N	3	1	1	2	3
Patient 3	Y	N	3	1	1	3	3
Patient 4	Y	N	3	2	2	2	3
Patient 5	N	N	1	2	2	1	1
Patient 5	N	N	3	1	1	1	3
Patient 7	Y	N	2	1	1	3	3
Patient 8	N	N	1	2	2	1	1
Patient 9	N	Y	3	2	2	3	3
Patient 10	N	N	2	2	2	2	2
Patient 11	Y	N	1	1	1	1	0
Patient 12	N	N	3	1	1	1	3
Patient 13	Y	N	3	2	2	0	3
Patient 14	N	N	1	0	1	0	1
Overall	Y = 6	Y = 1	3 (1–3)	1 (0–2)	1 (1–2)	1 (0–3)	3 (0–3)

DSA, donor-specific alloantibody; Y, yes; N, no; cg, chronic glomerular; ci, chronic interstitial; ct, chronic tubular; cv, chronic vascular; mm, matrix.

B cells before rituximab therapy, and the outcome of TG after rituximab therapy. Table 2 shows the results of the KB Banff classification. The patients did not have follow up KBs.

Donor-specific anti-HLA antibodies

DSAs were present in six cases (43%): this was anti-HLA class I alloantibodies in one case, and anti-HLA class II alloantibodies in five cases. They were present at the time of KBs, and remained so at last follow up. Of those patients with DSA, five out of six lost their graft as compared with only two out of eight patients without DSA ($P = 0.1$).

According to the ABCD tetrad (3), because we did not perform KBs on electron microscopy examinations, we could only classify our patients using ACD parameters: thus six patients were AD, and one patient was AC.

Renal parameters

At last follow up post-rituximab therapy (30 months: range 12–48), two patients had died at 1 and 3 years post-rituximab therapy with a functioning graft; the causes of deaths were pneumocystosis and suicide. Moreover, seven patients (50%) had lost their kidneys. This occurred in one patient at month 6 post-rituximab therapy, in two others at month 12, in one at month 19, and in the other three at month 26 (see Fig. 1). Those with a functioning allograft at follow up ($n = 7$) had stable creatinine when compared with the level before RTx therapy, i.e., 182 (110–260) vs. 161 (130–191) μmol/l (P = NS), and albuminuria had significantly decreased from 2660 (95–13430) to 500 (22–4150) mg/day ($P < 0.05$).

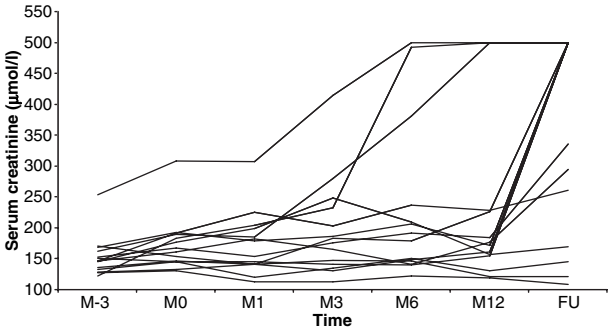

Figure 1 Outcome of serum creatinine following rituximab therapy. M, month; FU, follow up. Serum creatinine at 500 μmol/l equates return to dialysis.

Before rituximab therapy, albuminuria was 3350 (95–13430) mg/day; however, seven patients had nephrotic-range albuminuria, i.e., 5000 (3900–13430) mg/day. Overall, at 1, 3, 6, and 12 months after RTx therapy, and at last follow up, albuminuria had significantly decreased to 1040 (69–9800), 1255 (77–6000), 1400 (87–5700), 412 (23–10000), and 500 (22–2550) mg/day respectively ($P = 0.04$) (see Fig. 2). Of the seven patients with nephrotic-range albuminuria at baseline, i.e., 5000 (3900–13430) mg/day, at 1, 3 and 6 months after RTx therapy, albuminuria had decreased to 4260 (1000–9800), 2620 (600–6000), and 1800 (90–5700) mg/day respectively ($P = 0.03$).

With respect to albuminemia levels, which was 35 (27–43) g/l before rituximab therapy, this increased to 38 (23.6–47.5) g/l at 6 months post-rituximab therapy and to 38 (34.7–42.7) g/l at last follow up (P = NS). The response to rituximab therapy was not related to the number of rituximab doses.

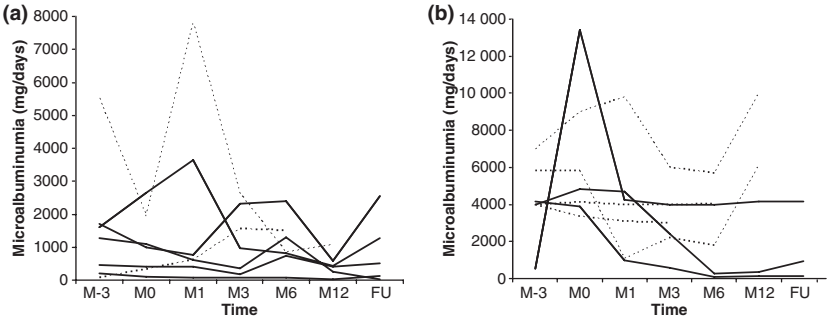

Figure 2 Outcome of microalbuminuria following rituximab therapy. M, month; FU, follow up. Dotted lines: patients that returned to dialysis; full lines: patients with a graft still functioning. (a) Those without nephrotic-range proleinumia at M0. (b) Those with nephrotic-range proleinumia at M0.

Cryoglobulinemia

Cryoglobulinemia was present in all patients before rituximab therapy although at low levels, i.e., 56 (10–810) mg/l. This was type II in four cases, type III in nine cases, and indeterminate in one case. However, with respect to CH50, C3, and C4 levels before rituximab therapy, these were within the normal ranges, i.e., 479 (431–663) UV, 0.86 (0.67–1.33) g/l, and 0.21 (0.15–0.32) g/l respectively. They did not significantly change throughout follow up post-rituximab therapy (data not shown). By 1 month post-rituximab therapy, cryoglobulinemia had decreased to 0 (0–9) mg/l, i.e., it remained positive in only one case. Cryoglobulinemia remained negative in almost all patients at 12-month follow up except in three patients (Table 3). Before rituximab therapy, for all patients, ANAs as well as ANCAs were negative, and serum immunoelectrophoresis showed no monoclonal components.

Safety/side-effects

Rituximab injections were very well tolerated, and were associated with no immediate side-effects. With respect to tolerance, up to the last follow up, there was no significant change in hemoglobin levels (Table 3) nor in leukocytes, polymorphonuclear, T cell or platelet counts (data not shown). There were no significant changes regarding plasma levels of total IgG, IgA, and IgM (Table 3). Rituximab therapy, as expected, had a dramatic impact upon B cell counts, i.e., these were 71 (21–664)/mm^3 before rituximab therapy, and then decreased to 0 mm^3 by 15 days 30 days, at 3, 6, and 12 months, and at last follow up post-rituximab therapy.

With respect to safety, i.e., infectious complications, four patients (28.5%) presented with infections: patient 2 presented with pneumococcal pneumopathy at 11 months after rituximab therapy, patient 4 presented with cytomegalovirus (CMV) disease at 11 months after rituximab therapy, patient 9 presented with disseminated herpes simplex type 2 infection at 5 months after rituximab therapy, and patient 14 presented with fatal pneumocystosis at 12 months after rituximab therapy. With respect to those patients who were HCV RNA positive at the time of rituximab therapy, these patients did not have significantly increased HCV RNA viremia or elevated levels of liver enzymes in the follow-up (data not shown).

Discussion

In this pilot study, we have shown that half of kidney-transplant patients with established transplant glomerulopathy respond to rituximab therapy.

Transplant glomerulopathy is a glomerular lesion that is unique to kidney allografts; it is characterized, by light microscopy, as capillary-wall widening, as double contours and is classified according to the Banff 2007 classification as cg 0 from 3. Transplant glomerulopathy evolves over time, progressing to double contours of glomerular basement membranes and an increase in mesangial matrix, sometimes with segmental sclerosis [4]. When transplant glomerulopathy lesions are observed on kidney allograft surveillance biopsies, even when allograft function is still normal, these silent lesions will translate to future worsening of allograft function and eventually to allograft loss [6]. Seron has recently reported that, on kidney allograft protocol biopsies, the association of interstitial fibrosis/ tubular atrophy (IF/TA) with transplant vasculopathy, subclinical rejection, or transplant glomerulopathy implies a poorer outcome than IF/TA with other histologic lesions [20]. At later stages, it can be very difficult to distinguish it from MPGN. MPGN could be secondary to chronic infectious processes such as chronic HCV infection [21]. In our series, seven patients had chronic HCV infection; of these, kidney biopsies performed just prior to rituximab therapy showed an aspect of MPGN in only one case: this patient also had donor-specific anti-HLA class II alloantibodies. Thus, we can assume that the glomerular lesions were more likely related to TG instead of HCV-related MPGN. However, recently Gloor *et al.* [7] have shown that, in a series of 582 patients of whom 55 had TG, the risk factors associated with the development of TG were acute rejection,

Table 3. Effects of rituximab therapy on kidney and hematologic parameters.

	M-3	M0	M1	M3	M6	M12	FU	*P* value
Serum creatinine (μmol/l)	146 (122–254)	161 (130–308)	154 (113–307)	176 (113–414)	164 (122–492)	159 (119–228)	190 (110–260)	NS
Creatinine clearance (ml/min)	46.8 (30.7–74.7)	40 (25.4–57)	39 (25–56)	39 (20–54)	39 (13–46)	40 (27–52)	35 (24–43.5)	NS
Albuminuria (g/d)	1695 (80–7000)	3350 (95–13430)	1040 (69–9800)	1255 (77–6000)	1400 (87–5700)	412 (23–10000)	500 (22–2550)	0.04
Albuminemia (*n*:7–38 g/l)	35 (30–43)	35 (27–43)	37 (28–44)	38 (24.5–42.5)	37 (31–44)	38 (23.6–47.5)	38 (34.7–42.7)	NS
Cryoglobulinemia (mg/l)	105 (0–341)	56 (10–810)	0 (0–37)	5 (0–950)	5 (0–309)	0 (0–177)	0 (0–153)	0.001
B lymphocytes (/mm^3)	ND	71 (21–664)	0 (0–9)	0 (0–7)	0 (0–30)	10 (0–55)	0 (0–195)	0.001
Hb (g/dl)	ND	12.6 (9–13.4)	12.4 (10.3–14.2)	13.3 (8.2–14.8)	12.4 (8.2–14.3)	13 (9.3–15.4)	12.4 (8.8–14.8)	NS
IgG (g/l)	ND	7.9 (4.6–21.1)	8 (3.8–15.2)	8.1 (3.6–15.3)	8.65 (3.5–14.7)	9.5 (5.6–16.3)	8.42 (5.8–13.7)	NS
IgA (g/l)	ND	2 (0.74–6.2)	2.16 (0.75–5.4)	2.05 (0.85–6.9)	2.3 (0.8–5.9)	2.3 (0.8–7.1)	2.45 (0.61–6.72)	NS
IgM (g/l)	ND	0.81 (0.33–3.65)	0.99 (0.44–1.73)	0.95 (0.5–1.72)	0.97 (0.52–1.75)	0.84 (0.4–1.6)	0.87 (0.54–1.35)	NS

Hb, hemoglobin; Ig, immunoglobulin; M, month; FU, follow up.

anti-HLA antibodies, especially anti-class II antibodies, and positivity for hepatitis C antibodies.

The Edmonton group have identified variable forms of transplant glomerulopathy according to the presence of four components (the so-called ABCD tetrad): antidonor antibodies (A), peritubular capillary basement-membrane multilayering (B), peritubular capillary C4d deposits (C), and GBM duplication (D) [3]; hence some patients might have all these (ABCD) or a combination of at least two components. However, at odds with this concept, recently, Akalin *et al.*, [1] in a cohort of 36 patients with transplant glomerulopathy, found that 89% and 64% of patients were C4d and DSA negative respectively. Because they did not perform electron microscopy studies on their kidney biopsies, it is difficult to say if less than 20% of their patients did not fulfill the ABCD tetrad. Because we did not perform electron microscopy studies in our series, only six patients were AD and one was AC. However, we could postulate that TG patients are either seen at various stages of their TG or, alternatively, that TG proceeds through various etiologies and, therefore, TG is the expression of various pathologies. Further investigations are warranted to answer these questions.

When a kidney-transplant patient presents with microalbuminuria, the policy at our center is to first do a work-up of albuminuria, and second, to perform a kidney allograft biopsy. We routinely assess ANAs as wells as ANCAs: these were negative in all our patients. We also assess the presence of cryoglobulinemia: this was present in all our patients although at a low level at 56 (10–810) mg/l; however, i) total complement levels as well as its C3 and C4 subfractions were within the normal ranges, ii) serum immunoelectrophoresis showed no monoclonal gammopathy, and iii) none of the kidney biopsies showed evidence of cryoglobulinemia-associated glomerulonephritis. Although we have no clear explanation for the presence of low levels of cryoglobulinemia, which is a common finding in kidney-transplant patients [18] we think that cryoglobulinemia is much more of a bystander than a culprit in the pathogenesis of transplant glomerulopathy.

When transplant glomerulopathy is diagnosed, therapeutic measures including protocols to reduce calcineurin-inhibitor exposure remain largely unproven [22]. At best it is suggested to maximize antiproteinuric treatment (e.g., angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers) [2] because such treatments are able to reduce proteinuria on native kidneys [23]. However, maximizing such therapies is not always easy and manageable in patients who have a single functioning kidney that is also suffering from transplant glomerulopathy injury. All our patients were already on maximal doses of antiproteinuric agents at the time of

transplant glomerulopathy diagnosis. Therefore, we wondered whether adding a treatment to target the B cells, which are implicated within the alloimmune response, might alter the natural course of their disease. Most of our patients were already on mycophenolate mofetil (MMF)-based therapy although this was not always associated with tacrolimus. However, even though the association of tacrolimus with MMF has been demonstrated efficient in reversing acute antibody-mediated rejection [24], there is still no evidence showing that this association is able to halt the progression of chronic allograft nephropathy.

Rituximab is a chimeric monoclonal antibody that is directed against the CD20 molecule present on the surface of pre-B and mature B cells, but is absent on the surface of plasmocytes [25]. Rituximab therapy has been shown to be efficient in inducing sustained remission in patients who have membranous glomerulopathy on native kidneys [26]. In the setting of kidney transplantation, rituximab therapy has been shown to reverse acute humoral rejection [27,28] but also to cure recurrent membranous glomerulopathy [17]. In kidney-transplant patients, when rituximab is used to treat acute humoral rejection, repeated allograft biopsies have consistently shown that it is associated with sustained B-cell depletion within the graft [29], even when there are B-cell aggregates [30].

Our pilot study was performed on 14 patients with TG; of these six had DSA. Kidney allograft biopsies showed i) no evidence of acute rejection, ii) glomerular lesions ranged from mild to severe, and iii) C4d deposits only occurred in one case. In 50% of the cases, rituximab therapy was associated with renal function stabilization and/or a decrease in albuminuria and this tended to be associated with the absence of DSA. However, we were unable to identify factors that could be associated either with or without a response of TG to rituximab therapy. Also, we assessed the presence or absence of B-cell lymphocytes on pre-rituximab kidney-allograft biopsies: this was not associated with the outcome.

Following rituximab therapy, we observed long-lasting B-cell lymphopenia, which is a common feature [31]. Because of this, and also because our kidney-transplant patients have T-cell function impairment as a result of chronic immunosuppression, this might explain why we observed unusual and severe infectious complications in four patients (28.5%). However, because we did have a control group, we cannot ascribe these infectious complications to rituximab therapy.

Transplant glomerulopathy is possibly related to various etiologies, including humoral-mediated lesions, e.g., DSA. This might explain why we found that TG rituximab therapy in half of our patients, when given on top of their T-cell directed immunosuppression, was able to halt the process, which was also associated in some patients with a dramatic decrease in albuminuria. However, safety is a concern because of infectious complications that occurred in some rituximab-treated patients, and we were not able to find predictors for rituximab therapy. However, those patients with DSA had a very poor outcome even though the *P*-value was not statistically significant ($P = 0.1$). A prospective trial on kidney-transplant patients with TG to compare rituximab with a placebo seems necessary.

In conclusion, rituximab therapy in kidney-transplant patients with transplant glomerulopathy is associated in the long-term with allograft function/stabilization in 50% of cases. Characterization of factors associated with this clinical response is mandatory; however, this can only be addressed in a prospective controlled trial.

Authorship

LR: designed the study, followed the patients and wrote the paper. CGF: did the pathologic analysis. MF: did the immunologic analysis. LM: collected the data. NK: did the statistical analysis and reviewed the paper.

References

1. Akalin E, Ames S, Sehgal V, *et al.* Intravenous immunoglobulin and thymoglobulin facilitate kidney transplantation in complement-dependent cytotoxicity B-cell and flow cytometry T- or B-cell crossmatch-positive patients. *Transplantation* 2003; **76**: 1444.
2. Cosio FG, Gloor JM, Sethi S, Stegall MD. Transplant glomerulopathy. *Am J Transplant* 2008; **8**: 492.
3. Sis B, Campbell PM, Mueller T, *et al.* Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant* 2007; **7**: 1743.
4. Maryniak RK, First MR, Weiss MA. Transplant glomerulopathy: evolution of morphologically distinct changes. *Kidney Int* 1985; **27**: 799.
5. Cosio FG, Grande JP, Larson TS, *et al.* Kidney allograft fibrosis and atrophy early after living donor transplantation. *Am J Transplant* 2005; **5**: 1130.
6. Cosio FG, Grande JP, Wadei H, Larson TS, Griffin MD, Stegall MD. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; **5**: 2464.
7. Gloor JM, Sethi S, Stegall MD, *et al.* Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant* 2007; **7**: 2124.
8. Mauiyyedi S, Pelle PD, Saidman S, *et al.* Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001; **12**: 574.

9. Vongwiwatana A, Gourishankar S, Campbell PM, Solez K, Halloran PF. Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy. *Am J Transplant* 2004; **4**: 124.
10. Gloor J, Cosio F, Lager DJ, Stegall MD. The spectrum of antibody-mediated renal allograft injury: implications for treatment. *Am J Transplant* 2008; **8**: 1367.
11. Basse G, Ribes D, Kamar N, *et al.* Rituximab therapy for de novo mixed cryoglobulinemia in renal transplant patients. *Transplantation* 2005; **80**: 1560.
12. Gossmann J, Scheuermann EH, Porubsky S, Kachel HG, Geiger H, Hauser IA. Abrogation of nephrotic proteinuria by rituximab treatment in a renal transplant patient with relapsed focal segmental glomerulosclerosis. *Transpl Int* 2007; **20**: 558.
13. Kamar N, Faguer S, Esposito L, *et al.* Treatment of focal segmental glomerular sclerosis with rituximab: 2 case reports. *Clin Nephrol* 2007; **67**: 250.
14. Kuypers DR, Lerut E, Claes K, Evenepoel P, Vanrenterghem Y. Recurrence of light chain deposit disease after renal allograft transplantation: potential role of rituximab? *Transpl Int* 2007; **20**: 381.
15. Hristea D, Hadaya K, Marangon N, *et al.* Successful treatment of recurrent focal segmental glomerulosclerosis after kidney transplantation by plasmapheresis and rituximab. *Transpl Int* 2007; **20**: 102.
16. Meyer TN, Thaiss F, Stahl RA. Immediate versus long-term effect of rituximab in recurrent focal segmental glomerulosclerosis. *Transpl Int* 2008; **21**: 1102.
17. Weclawiak H, Ribes D, Guilbeau-Frugier C, *et al.* Relapse of membranous glomerulopathy after kidney transplantation: sustained remittance induced by rituximab. *Clin Nephrol* 2008; **69**: 373.
18. Faguer S, Kamar N, Boulestin A, *et al.* Prevalence of cryoglobulinemia and autoimmunity markers in renal-transplant patients. *Clin Nephrol* 2008; **69**: 239.
19. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
20. Seron D. Risk factors associated with the deterioration of renal function: the role of protocol biopsies. *Prilozi* 2007; **28**: 291.
21. Kamar N, Izopet J, Alric L, Guilbeaud-Frugier C, Rostaing L. Hepatitis C virus-related kidney disease: an overview. *Clin Nephrol* 2008; **69**: 149.
22. Najafian B, Kasiske BL. Chronic allograft nephropathy. *Curr Opin Nephrol Hypertens* 2008; **17**: 149.
23. Bakris GL. Slowing nephropathy progression: focus on proteinuria reduction. *Clin J Am Soc Nephrol* 2008; **3** (Suppl 1): S3.
24. Pascual M, Saidman S, Tolkoff-Rubin N, *et al.* Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. *Transplantation* 1998; **66**: 1460.
25. Taylor RP, Lindorfer MA. Immunotherapeutic mechanisms of anti-CD20 monoclonal antibodies. *Curr Opin Immunol* 2008; **20**: 444.
26. Ruggenenti P, Chiurchiu C, Brusegan V, *et al.* Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 2003; **14**: 1851.
27. Becker YT, Becker BN, Pirsch JD, Sollinger HW. Rituximab as treatment for refractory kidney transplant rejection. *Am J Transplant* 2004; **4**: 996.
28. Faguer S, Kamar N, Guilbeaud-Frugier C, *et al.* Rituximab therapy for acute humoral rejection after kidney transplantation. *Transplantation* 2007; **83**: 1277.
29. Steinmetz OM, Lange-Husken F, Turner JE, *et al.* Rituximab removes intrarenal B cell clusters in patients with renal vascular allograft rejection. *Transplantation* 2007; **84**: 842.
30. Lehnhardt A, Mengel M, Pape L, Ehrich JH, Offner G, Strehlau J. Nodular B-cell aggregates associated with treatment refractory renal transplant rejection resolved by rituximab. *Am J Transplant* 2006; **6**: 847.
31. Abdallah KO, Prak ET. B cell monitoring of transplant patients treated with anti-CD20. *Clin Transpl* 2006; 427.

