LETTER TO THE EDITORS

Rituximab as monotherapy for the treatment of chronic active antibody-mediated rejection after kidney transplantation

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Dear Editors,

Chronic active antibody-mediated rejection (caAMR) is a major cause of allograft loss after kidney transplantation [1]. The BANFF 2013 classification redefined caAMR by the presence of donor-specific anti-HLA antibodies (DSA) together with immunohistopathological evidence for active vascular lesions of the endothelium (C4d deposits, glomerulitis, and peritubular capillaritis) as well as evidence of chronic tissue injury (transplant glomerulopathy, peritubular capillary basement membrane multilayering, or arterial intimal fibrosis) [2,3]. Humoral immunity, detected by the presence of DSA, and B cells are considered pivotal in the development of caAMR. Gosset *et al.* [1] showed that circulating DSA are responsible for accelerated allograft fibrosis independently of acute AMR. Recent evidence suggested that B cells also mediate chronic allograft rejection independently of DSA [4] and that rituximab, a chimeric IgG1 antibody targeting human CD20, impairs B-cell regulation of T cells [5–7]. However, the efficacy of rituximab in patients with caAMR remains controversial [8].

We retrospectively analyzed 12 kidney transplant recipients followed at our institution between January 2007 and December 2012 who were diagnosed with biopsy-proven caAMR (Table 1). All patients were treated with a regimen of two doses of rituximab (375 mg/m^2) at 1-week interval, as monotherapy, together with a transient increase of 20 mg/day oral prednisone, progressively tapered to 5 mg at 3 months. Peripheral B-cell depletion (<10 B cells/μl) after rituximab administration was confirmed by flow cytometry. In seven of 12 patients, there was a decrease in DSA levels below 2000 mean fluorescence intensity (MFI) (Luminex, Austin, TX, USA) or a >75% DSA level decrease after rituximab (Fig. 1a). Serum creatinine and proteinuria levels were not significantly different after rituximab. Regarding active antibody-mediated vascular lesions, four of six patients with positive C4d deposits, six of eight with peritubular capillaritis, and three of four with glomerulitis had a decrease in the activity score at 1-year postrituximab (Fig. 1b). However, chronic histological lesions did not improve 1 year after rituximab (Fig. 1c). We further analyzed clinical responders defined as patients who improved or maintained stable kidney function at 12 months postrituximab (<20% change of the baseline creatinine) with a decrease in DSA titers below 2000 MFI or >75% DSA titers decrease. The results showed significantly more C4d deposition in patients with persistent DSA before and after rituximab (Fig. 2a,b), whereas no significant differences were found between clinical responders and nonresponders regarding capillaritis and glomerulitis (Fig. 2c,d).

We performed CD20 and CD45 staining on all kidney allograft biopsies before and after rituximab. The results showed a significant reduction in CD20$^+$ B cells in the kidney allograft at 1 year (Fig. 3a,b). Interestingly, the absolute number of CD45$^+$ was not changed (Fig. 3c,d). Peripheral B-cell depletion (<10 B cells/μl) was present only in five of 12 patients after 1 year, and absolute B-cell numbers were not different between responders and nonresponders (data not shown). These results demonstrated that CD20$^+$ B cells were targeted in the kidney allograft, although complete long-term depletion was not achieved.

Evidence for successful management of caAMR is currently lacking. The first randomized trial of

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Table 1. Baseline clinical and biological data of all patients.

Patient	Age	Sex	Hypertension	Diabetes mellitus	2d TX	Anterior ACR	IS regimen	Interval TX-DSA (months)	Interval DSA-RTX (months)
1	47	M	Yes	No	No	No	Tac/MMF/Pred	6	1
2	31	M	Yes	No	No	Yes	SRL/MMF/Pred	14	2
3	21	F	No	No	No	Yes	Tac/MMF/Pred	20	0
4	30	F	Yes	No	No	No	CsA/MMF/Pred	30	3
5	20	M	No	No	No	Yes	Tac/MMF	132	0
6	40	F	Yes	No	No	No	SRL/MMF/Pred	84	1
7	61	F	Yes	No	No	No	Tac/MMF	74	3
8	20	F	Yes	No	No	Yes	Tac/MMF/Pred	7	1
9	55	M	Yes	No	Yes	No	Tac/MMF/Pred	0	20
10	46	M	No	Yes	No	No	Tac/MMF	58	4
11	39	M	Yes	No	No	Yes	Tac/MMF	ND	9
12	22	F	Yes	Yes	No	Yes	Tac/MMF/Pred	26	2

Patient	Serum creatinine µmol/l before RTX	Serum creatinine µmol/l after RTX	Proteinuria Prot/creat g/mol before RTX	Proteinuria Prot/creat g/mol after RTX	Likely non-compliant	Tac (µg/l) CsA (µg/l) level at diagnosis
1	149	123	2770	530	N	7.6
2	115	120	800	120	N	5.8
3	125	127	400	300	Y	<2
4	215	228	100	190	Y	239
5	90	107	10	0	N	5.5
6	160	149	380	147	N	–
7	112	113	150	0	N	6.2
8	178	290	34	90	Y	6.2
9	118	119	2360	3500	N	10
10	100	141	140	3000	N	7
11	180	197	700	770	N	5.4
12	240	291	130	430	Y	5.5

MMF, mycophenolate mofetil; ND, not defined; Pred, prednisone; Tac, tacrolimus; Csa, cyclosporine; SRL, sirolimus. TX, transplantation date; DSA, donor-specific antibodies; RTX, rituximab; ACR, acute cellular rejection.

eculizumab therapy for caAMR failed to show clinically relevant efficacy on kidney function, DSA titers, and chronic active histopathological lesions [9]. More recently, Moreso *et al.* [10] published a second randomized trial comparing patients with transplant glomerulopathy and DSA receiving four cures of intravenous immunoglobulins (IVIG) followed by one infusion (375 mg/m^2) of rituximab compared to placebo, but no improvement in histological lesions, DSA titers, and kidney function was observed. Although both studies were underpowered, they demonstrated that untreated patients did not improve chronic active histological lesions and maintained high-titer DSA.

Despite the negative results of the two randomized trials, there is some evidence suggesting that B-cell depletion is important in the management of caAMR. Thus, patients highly sensitized or receiving ABO-incompatible transplants were more protected against chronic rejection if treated with rituximab [11,12]. In the setting of acute AMR and intensive immunosuppression regimen, rituximab seems also to be beneficial [5,13,14]. In the present series, rituximab administration was associated with a reduction in DSA levels and active histopathological lesions in most patients, although this did not translate in an improvement of allograft function, which was also confirmed by the absence of improvement of chronic lesions in 1-year control biopsies. In the EudraCT trial, rituximab was administered only once, up to 6 months after that the diagnosis was made resulting in no improvement of DSA or histological lesions [10]. The results of the prospective multicentric randomized trial (NCT00307125), aiming to compare the use of rituximab versus placebo for the treatment of

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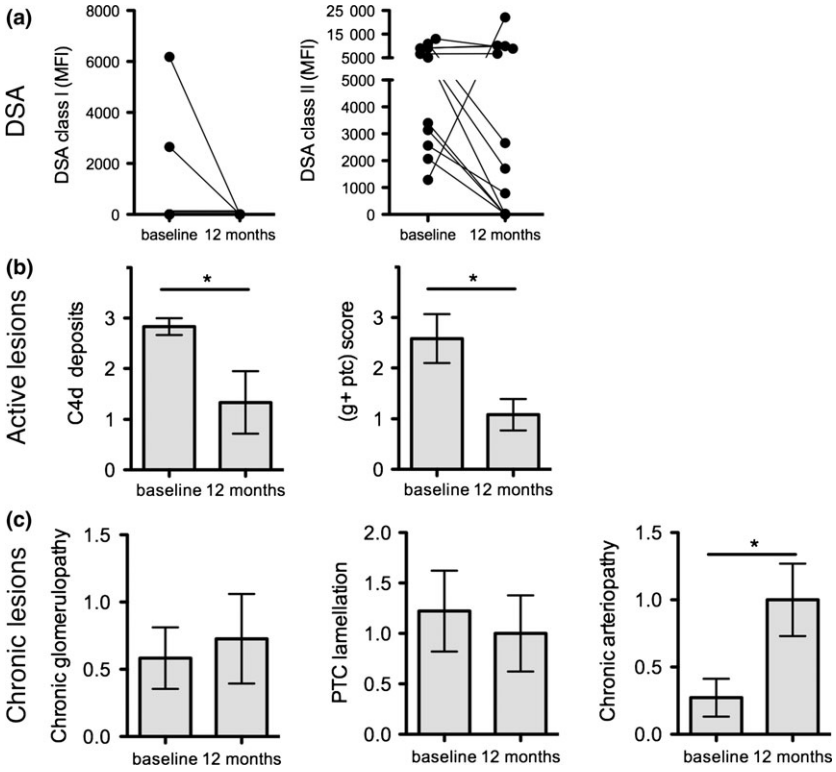

Figure 1 (a) Mean fluorescence intensity (MFI) of *de novo* class I and II donor-specific antibodies (DSA) before and 12 months after rituximab. (b) Active histological lesions including capillary C4d deposits and microvascular inflammation score calculated as the combination score of glomerulitis (g) and peritubular capillaritis (ptc). (c) Chronic histopathological lesions including chronic glomerulopathy score, peritubular capillary (PTC) lamellation score, and chronic arteriopathy score. All renal biopsies were evaluated by the same experienced pathologist. Two-tailed Student's *t* tests were used. Mean and standard deviations are shown. **P* value <0.05.

de novo early DSA in kidney allograft recipients over a 3-year period time, are therefore expected with interest. Finally, it must be also emphasized that future protocols should consider the possibility to reinfuse rituximab every 6 months as B-cell depletion was found to be significantly reduced but not complete 1 year after rituximab.

The importance of chronic active T-cell-mediated rejection in caAMR should not be neglected as T helper cells are key in priming B cells. Earlier studies showed that reversal of DSA can be achieved in the absence of a "desensitization regimen", but in general, this was associated with some rescue therapy based on tacrolimus and mycophenolate mofetil switch and corticosteroids [15,16]. Furthermore, allograft survival can be significantly improved after *de novo* DSA detection if levels of tacrolimus are maintained above 5.3 ng/ml [17]. In our series, patients received transiently more oral prednisone, which possibly acted synergistically with rituximab against active lesions, although it must be acknowledged that, in the absence of an untreated control group, the interpretation of the data must be taken with caution.

Tocilizumab and C1-inhibitor have also been studied for caAMR. Thirty-six patients who were refractory to rituximab and IVIG treatment received tocilizumab, an anti-IL-6 receptor monoclonal antibody, as rescue therapy, which resulted in reduction in DSA titers and in stabilization of allograft function at 2 years [18]. Because rituximab depletes $CD20^+$ precursor B cells and tocilizumab disrupts the IL-6 supplying niches important for Th1/T follicular differentiation and long-lived plasma cells, a synergic beneficial action of both antibodies could hypothesized as in Castleman's disease [19]. Recently, plasma-derived human C1-inhibitor (20 UI/kg/twice weekly), an inhibitor which targets the classical complement pathway, was successfully administered for caAMR prevention in highly sensitized patients [20,21]. Thus, in the future, nonresponders to rituximab and/or IVIG may be candidates for combination therapy of B-cell-depleting agents with complement inhibitors or tocilizumab.

At our center, we do not perform routine protocol biopsies, so that we did not have the possibility to select well-matched controls. Even if most patients responded to rituximab, active lesions, B cells, and, in some cases, DSA remained present. This was also reported by Gupta *et al.* [22] who targeted B cells using a combination of rituximab, IVIG, and bortezomib, showing that 48% of the patients with late AMR were nonresponders [22]. In the future, more detailed analysis is warranted on the potential relationship between DSA, mean fluorescence intensity, HLA types (IgG-IgM-IgA), subclasses (IgG 1/2/3/4), HLA class I or II specificity, epitope specificity, complement binding (C1q) DSA ability, and clinical

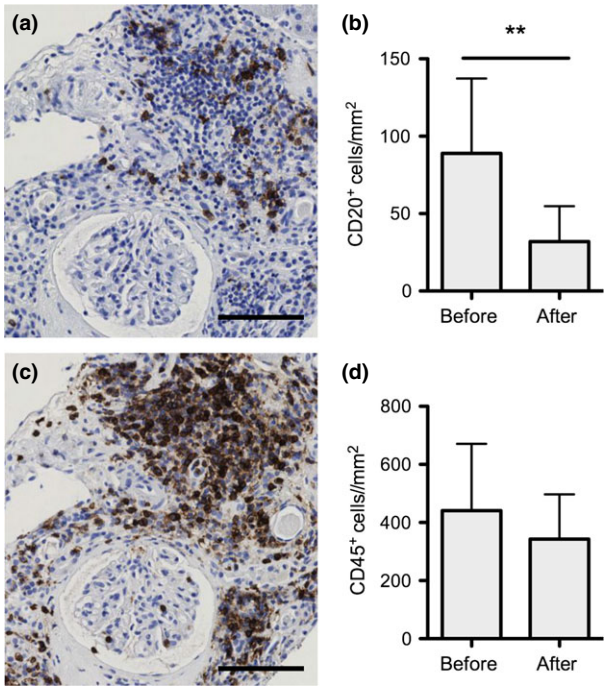

Figure 2 (a) Representative CD20 staining. (b) Allograft CD20-positive cells were stained, slides were scanned (Zeiss, Oberkochen, Germany), and cells were counted before and one year after rituximab. (c) Representative CD45 staining. (d) Allograft CD45-positive cells were stained, slides were scanned, and cells were counted before and one year after rituximab. Immunohistochemistry was performed on paraffin-fixed tissue for mouse anti-human CD20 (1/400, clone L26; Novocastra/Leica, Wetzlar, Germany) and mouse anti-human CD45 (1/1000, clone 2B11; DAKO, Ely, UK) with automat (Ventana) per our clinical standardized protocol. The slides were thereafter scanned (Mirax; Zeiss), and cells were quantified using the DIH Image analysis software and the measure stained cells Algorithm (Slidepath software; Leica). ***P* value <0.01.

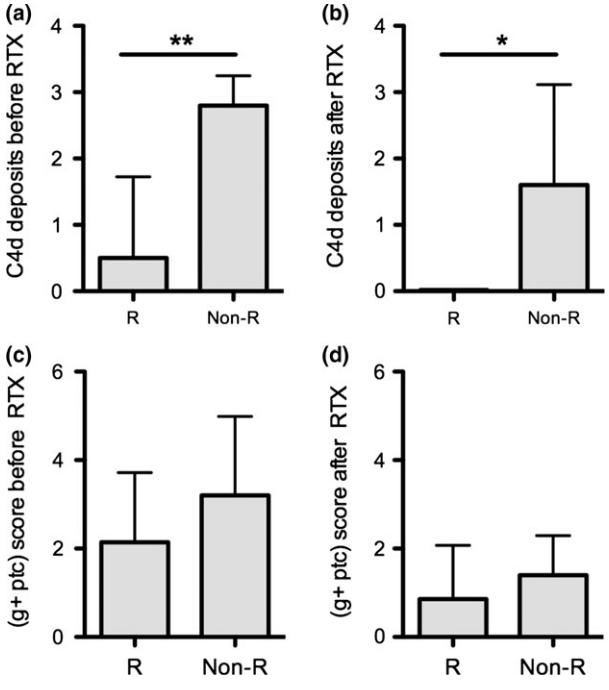

Figure 3 (a) C4d deposition score was compared in clinical responders and nonresponders before (a) and 12 months after rituximab. (b) The combined peritubular capillaritis and glomerulitis score was assessed in both group before (c) and 12 months after (d) rituximab. **P* value <0.05. ***P* value <0.01.

responses to rituximab. Moreover, distinctions between patients with pre-existing DSA and *de novo* DSA would also be interesting [23]. Overall, it would be important to better define which transplant recipients can benefit from rituximab, alone, or in combination with other therapies.

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Conflicts of interest

The authors have declared no conflicts of interest.

Ethical protocol

This study was reviewed and accepted by the "Commission cantonale d'éthique de la recherche sur l'être humain" (CER-VD, protocol 2017-01077).

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