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ORIGINAL ARTICLE

Rabbit anti-human thymocyte immunoglobulin for the rescue treatment of chronic antibody-mediated rejection after pediatric kidney transplantation

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Abstract
Background Chronic antibody-mediated rejection (cAMR) is the leading cause of late kidney graft loss, but current therapies are often ineffective. Rabbit anti-human thymocyte immunoglobulin (rATG) may be helpful, but its use is virtually undocumented.
Methods Data were analyzed retrospectively from nine pediatric kidney transplant patients with cAMR were treated with rATG (1.5 mg/kg × 5 days) at our center after non-response to pulsed prednisolone, intravenous immunoglobulin, rituximab, and increased immunosuppressive intensity (including switching to belatacept in some cases), with or without bortezomib.
Results The median time from diagnosis to cAMR was 179 days. rATG was started 5–741 days after diagnosis. Median estimated glomerular filtration rate (eGFR) increased from 40 mL/min/1.73 m^2 when rATG was started to 62 mL/min/1.73 m^2 9 months later ($p = 0.039$). Four patients showed substantially higher eGFR after 9 months and 2 patients showed a small improvement; eGFR continued to decline in 3 patients after starting rATG. No grafts were lost during follow-up. At last follow-up, donor-specific antibodies (DSAs) were no longer detectable in 4 out of 8 patients for whom data were available, median fluorescence intensity had decreased substantially in 1 out of 8 patients; anti-HLA DQ DSAs persisted in 2 out of 8 patients. No adverse events with a suspected relation to rATG, including allergic reactions, leukocytopenia or infections, were observed in any of the patients.
Conclusions In this small series of patients, rATG appears a promising treatment for unresponsive cAMR. Further evaluation, including earlier introduction of rATG, is warranted.

Keywords Antibody-mediated rejection · DSA · Rabbit anti-thymocyte immunoglobulin · ATG · rATG · Thymoglobulin

Introduction

The key role of antibodies in the immunological response to organ transplantation, which has been neglected for decades, has recently become more fully appreciated. Development of antibodies against alloantigens is central to the effector mechanisms of the adaptive immune system, and can culminate in chronic antibody-mediated rejection (cAMR) [1]. cAMR can follow a variable clinical course, with either subclinical or clinically evident proteinuria and gradual loss of graft function over several years [2, 3], but 15–20% of adult kidney grafts fail within the first year after diagnosis of AMR [4] and it is the leading cause of late kidney graft loss [5, 6]. The North American Pediatric Renal Trials and Collaborative Studies reported in 2014 that 50.7% of all graft failures are caused by rejection, with chronic rejection accounting for 35.8% of these failures, and it is likely that many of these were in fact AMR [7]. Data on the frequency of cAMR in children are lacking, but their relatively naïve immune system could potentially place them at even greater risk, compounded in adolescents by a high risk for non-adherence to the immunosuppressive regimen [8].

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Chronic AMR has a complex pathophysiology. Donor-specific antibodies (DSAs) against human leukocyte antigen (HLA) play a central role in chronic graft deterioration [9], and post-transplant development of DSAs is associated with a higher risk for AMR and graft loss in adults [9, 10] and children [11]. cAMR is notoriously difficult to treat, particularly once it has progressed to transplant glomerulopathy, and few controlled studies have been conducted in this area [12]. In addition to maintaining or increasing the intensity of maintenance immunosuppression, treatment is aimed at eliminating the antibodies (notably DSAs) that have induced AMR and, second, to inhibit production of further antibodies by targeting of B-cells and plasma cells (the source of DSAs). A further approach is to inhibit the complement cascade. Removal of circulating DSAs is typically attempted using intravenous immunoglobulin G (IVIG) [13]. The addition of plasmapheresis, or immunoadsorption in the event of nonresponse to plasmapheresis, can be helpful. Randomized controlled trials in adults have shown that plasmapheresis removes 50% of antibodies, but rebound occurs and administration of IVIG increases response rate to 50–90% [14, 15]. However, extracorporeal therapy is a therapeutic challenge in small children. To deplete B-cells, the chimeric monoclonal anti-CD20 antibody rituximab is an established option for refractory AMR [6] but CD20-negative plasma cells are unaffected. Where cAMR does not respond to depletional agents, newer agents such as plasma-derived C1 esterase inhibitor [16] and the anti-interleukin-6 receptor antagonist tocilizumab [17] have shown promising results.

Rabbit anti-human thymocyte immunoglobulin (rATG) has a well-established role in induction therapy, but some centers also use rATG to suppress antibody production after diagnosis of cAMR following kidney [13] or heart [18] transplantation. In recent years, it has been recognized that rATG not only depletes T-cells—including the helper T-cells required to elicit a B-cell response to antibodies—but may also suppress memory and switch memory B-cell subpopulations [19–23]. The use of rATG to treat cAMR, however, is based only on tangential evidence from prophylactic applications: namely, that it prevents production of DSAs de novo post-transplant [24] and that it contributes to the effectiveness of pre-transplant desensitization protocols [25].

To our knowledge, experience of treating cAMR with rATG is virtually undocumented. We report here a retrospective analysis of nine pediatric kidney transplant patients in whom rATG was given at our center after a poor response to other therapeutic options.

Materials and methods

This was a retrospective review of cases from a single center (Department of Pediatric Nephrology, Hannover Medical School, Germany). Cases were identified from all patients who regularly attended the pediatric nephrology outpatient department between 2006 and 2016. Data were collected from medical records. As each case was classified as an "individual healing attempt" according to German law, no ethics approval was required.

During the study period, all patients received basiliximab induction therapy. The initial maintenance regimen comprised cyclosporine (CsA) with prednisolone. At week 4 post-transplant, the CsA trough concentration was halved and everolimus was started. If protocol biopsy at month 6 showed normal histology, prednisolone was tapered and discontinued [26]. Details of the immunosuppressive regimen, including dosing and target concentrations, have been published previously [27].

The panel reactive antibody (PRA) value was defined as the percentage of panel cells that reacted with patient serum in the complement-dependent cytotoxicity screening. HLA matches were calculated for the loci HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ at the time of organ allocation. For high-resolution typing, CTS-Sequence kits (Heidelberg, Germany) and Olerup-SSP kits (Saltsjöbaden, Sweden) were used. HLA antibodies were measured before engraftment and at least annually post-transplant, or if the glomerular filtration rate (GFR) decreased by more than 20% from baseline. Measurements were made using the LABScreen single-antigen beads Luminex kit (One Lambda, Canoga Park, CA, USA) which uses single HLA-coated beads and enables identification of IgG alloantibody specificities against HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 antigens. Because no clinically validated cut-off for the Luminex assay is recommended by the provider company, a mean fluorescence intensity of >1,000 was used to define the cut-off for antibody positivity as this appears to be predictive for adverse outcomes, including cAMR, following kidney transplantation [28–30]. Renal biopsy was performed if a patient was positive for DSAs according to this definition, and if estimated glomerular filtration rate (eGFR) decreased by >20%. cAMR was diagnosed according to the Banff 2013 criteria, combining pathological diagnosis and evidence for DSAs [31]. If cAMR was diagnosed, the initial treatment was six pulses of prednisolone (6 pulses of 300 mg/m^2 body surface area [BSA]), weekly courses of IVIG starting on day 2 (1 g/kg × 4 doses), and between one and four monthly infusions of rituximab starting on day 6 (375 mg/m^2 BSA), as described by Billing et al. [32]. If serum creatinine increased rapidly, the patient was given six sessions of immunoadsorption. If the patient had low trough concentrations of everolimus (<4 μg/l) or CsA (<60 mg/l) the dose was increased and, in steroid-free patients, prednisolone was re-started at a dose of 3 mg/m^2 BSA. If trough concentrations of everolimus and CsA were adequate, CsA was changed to tacrolimus (trough concentration 8–10 mg/l) and/or everolimus was changed to mycophenolate mofetil (MMF). In cases

of non-adherence to the calcineurin inhibitor (CNI) regimen—considered a likely cause of DSA development—patients were switched from CNI therapy to belatacept (with everolimus) if the patient agreed to continuous intravenous therapy [33]. Adherence was assessed by a combination of self-reporting and physician assessment. If graft function did not improve and the mean fluorescence values of DSAs did not decline in response to these interventions, bortezomib therapy was applied, as described by Walsh et al. [34]. Where graft function still showed progressive deterioration, or where the response was considered inadequate, rATG (Thymoglobulin®) was administered via a peripheral line at a dose of 1.5 mg/kg over 4 h for 5 consecutive days (total dose 7.5 mg/kg).

Renal function was assessed by eGFR using the Schwartz formula [35]. The ratio of urinary albumin/creatinine was measured as a marker for proteinuria. Renal data are reported for the 9 months before diagnosis of cAMR, at the time of cAMR diagnosis and the time of rATG introduction, and for the 9 months after start of rATG therapy. Data are shown only for post-transplant measurements, and thus 9 months of pre-rATG values are not included if cAMR was diagnosed less than 9 months after transplantation. Available data were too sparse for meaningful analysis at later time points, i.e., beyond 9 months.

Every 3 months, patients were monitored for Epstein–Barr virus (EBV), cytomegalovirus, and BK polyomas virus by polymer chain reaction (LightCycler®; Roche Diagnostics, Basel, Switzerland). Full blood counts were performed daily for 1 week post-transplant, then every 2 weeks. CD3 counts were not monitored.

As data were not normally distributed, results are primarily presented as median values and ranges. Mean values and standard variations were also determined. Statistical analysis was performed using the Mann–Whitney U test and $p < 0.05$ was defined as significant.

Results

Patient population and immunosuppression

Nine patients were assessed (3 female, 6 male), all of whom had received a first kidney transplant. Two patients received a living-donor graft (patients #2 and #6; patient #6 was transplanted pre-emptively). Key characteristics are shown in Table 1. All patients were followed for 9 months after the start of rATG therapy. All patients received induction with basiliximab, with an initial maintenance regimen comprising CsA and steroids. All patients were switched to low-exposure CNI therapy with CsA and everolimus at week 4 post-transplant (Table 2). In 1 patient, CsA was later switched to extended-release tacrolimus because of an episode of acute cellular rejection; the same patient was converted from everolimus to sirolimus to enable once-daily administration.

Diagnosis and management of cAMR

The median time between transplantation and diagnosis of cAMR was 179 days. Patients were a median age of 14.7 years at the time of diagnosis (Table 1). The pathological findings confirming diagnosis of active cAMR are summarized in Table 3. None of the patients underwent a repeat biopsy. Thrombotic microangiopathy was excluded in each case based on findings from clinically-indicated biopsies. In patients #4, #8, and #6, cellular rejection of BANFF grade Ia was also diagnosed, with cellular rejection BANFF grade Ib in patient #9. One patient (#8) did not fully meet the Banff 2013 criteria, as Luminex testing of DSAs was not available at the time of diagnosis. All 9 patients received pulsed prednisolone, four IVIG infusions, and rituximab. Additionally, 2 patients (#1 and #8) received immunoadsorption and bortezomib was given to 8 patients (all except patient #8, in whom post-transplant Luminex data on DSAs were unavailable).

Following diagnosis of cAMR, the immunosuppression regimen was modified in a tailored manner, by increasing CNI exposure or switching to tacrolimus, increasing mammalian target of rapamycin (mTOR) inhibitor exposure or, less frequently, introducing MMF (Table 2). Three patients (#2, #4, and #5) switched from CNI therapy to belatacept, all with everolimus. All 3 patients were considered non-adherent, and were EBV IgG-positive. The remaining 6 patients (patients #1, #3, #6, #7, #8, and #9) were considered to be adequately adherent to the immunosuppressive regimen.

Before the introduction of rATG, these interventions led to an improvement in eGFR in 3 patients (#4, #5, and #9), whereas eGFR continued to deteriorate in the other 6 patients. However, as the improvement in eGFR was only moderate in patients #4, #5, and #9, the decision was made to initiate rATG therapy in all cases. All patients received the planned five doses (total dose 7.5 mg/kg). The delay between diagnosis of cAMR and introduction of rATG ranged from 5 days to 741 days (Table 1). All patients received the full cumulative dose of rATG (7.5 mg/kg).

Donor-specific antibodies

None of the patients had pre-formed DSAs against HLA at the time of transplantation. At the time of cAMR diagnosis, 5 patients had DSAs against HLA-DQ, 1 against HLA-A, 1 against HLA-B, and 1 against both HLA-DQ and HLA-A (Table 1). At last follow-up, DSAs were no longer detectable in 4 patients. The median fluorescence intensity (MFI) level remained largely unchanged in 3 patients (#1, #3, and #7), all of whom had anti-HLA-DQ DSAs, and decreased to a clinically relevant extent [36] in 1 patient (#4; Table 1).

Table 1 Patient characteristics, treatment, and donor-specific antibodies (DSAs) status

Patient	#1	#2	#3	#4	#5	#6	#7	#8	#9
Gender	Female	Male	Female	Male	Male	Male	Female	Male	Male
Weight, kg	40.1	45.7	5.3	116.5	45.0	52.4	8.7	40.0	55.9
Primary disease leading to end-stage renal disease	Bartter–Syndrome	Cat Eye Syndrome	ARPKD	Renal dysplasia	Denys–Drash syndrome	Joubert Syndrome	Atypical HUS	FSGS	Renal dysplasia
Donor type	DD	LD	DD	DD	DD	LD	DD	DD	DD
Donor age, years	41	35	7	6	3	46	20	52	2
HLA mismatches, *n*	5	5	8	6	4	3	4	2	7
PRA at time of transplant, %	16	73	9	6	0	0	55	0	0
Age at diagnosis of cAMR, years	14.7	15.5	3.0	15.6	15.3	14.4	1.8	12.5	14.0
eGFR at diagnosis of cAMR, mL/min/1.73 m^2	54	67	57	40	28	64	47	45	48
Time from transplant to diagnosis of cAMR, days	407	179	871	61	72	761	4469	25	101
Treatment before start of rATG	Pulsed prednisolone IVIG × 4 Rituximab × 4 Immunoadsorption Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib	Pulsed prednisolone IVIG × 4 Rituximab × 4 Immunoadsorption	Pulsed prednisolone IVIG × 4 Rituximab × 4 Bortezomib
Time from transplant to start of rATG, days	469	214	1,020	90	163	923	4,828	766	106
De novo DSAs (MFI)[a]									
Time of treatment	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Time of cAMR diagnosis	DQ7 (MFI 20006)	A23 (MFI 11474, A24 (9229), DQ2 (MFI 3535)	DQ6 (MFI 6692)	DQ5 (MFI 20020)	A29 (MFI 1961)	B60 (MFI 1556)	DQ3 (MFI 9624)	Luminex data not available	DQ5 (MFI 3562) Negative
Last follow-up after rATG	DQ7 (MFI 18705)	Negative	DQ2 (MFI 11306)	DQ5 (MFI 1561)	Negative	Negative	DQ3 (MFI 7777)	Luminex data not available	

ARPKD autosomal recessive polycystic kidney disease, *cAMR* chronic antibody-mediated rejection, *DD* deceased donor, *DSAs* donor-specific antibodies, *eGFR* estimated glomerular filtration rate, *FSGS* focal segmental glomerulosclerosis, *HLA* human leukocyte antigen, *HUS* hemolytic–uremic syndrome, *IVIG* intravenous immunoglobulin G, *LD* living donor, *MFI* median fluorescence intensity, *PRA* panel reactive antibodies, *rATG* rabbit antithymocyte globulin,

[a] Based on the most recent assessment before the event

Table 2 Maintenance immunosuppression

Patient	#1	#2[a]	#3	#4[a]	#5[a]	#6	#7	#8	#9
CNI trough concentration, ng/mL									
Time of transplant	CsA 211	CsA 125	CsA 165	CsA 95	CsA 230	CsA 148	CsA 244	CsA 197	CsA 239
Time of cAMR diagnosis	CsA 37	CsA 53	TAC 9.2	CsA 57	CsA 52	CsA 75	CsA 75	CsA 68	CsA 82
Time of rATG initiation	CsA 62	CsA 52	TAC 4.5	TAC 7.8	CsA 65	TAC 7.8	TAC 8.0	CsA 78	CsA 69
Month 9 after rATG	TAC 8.9	–	TAC 2.9	–	–	–	TAC 6.0	TAC 13.3	CsA 35
mTOR inhibitor concentration, ng/mL									
Time of transplant	–	–	–	–	–	–	–	–	–
Time of cAMR diagnosis	EVR 2.3	EVR 3.2	EVR 4.2	EVR 7.1	–	EVR 3.6	–	SIR 2.6	EVR 2.7
Time of rATG initiation	EVR 3.4	EVR 6.0	EVR 6.8	EVR 9.8	EVR 7.9	EVR 3.3	–	SIR 5.5	EVR 2.8
Month 9 after rATG	–	–	EVR 3.8	EVR 5.4	EVR 8.1	EVR 5.2	–	SIR 9.3	EVR 2.2
MMF dose, g/day									
Time of transplant	–	–	–	–	–	–	0.5	0.5	–
Time of cAMR diagnosis	–	–	–	–	2.0	–	–	–	–
Time of rATG initiation	–	–	–	–	2.0	–	–	–	–
Month 9 after rATG	1.5	–	–	–	2.0	–	–	–	–
Steroids, mg/day									
Time of transplant	20	17.5	15	50	17.5	25	15	15	25
Time of cAMR diagnosis	10	5	2.5	7.5	5	5	5	15	5
Time of rATG initiation	10	5	2.5	7.5	5	5	5	12.5	5
Month 9 after rATG	20	5	2.5	7.5	5	5	5	10	5

BAS basiliximab, *cAMR* chronic antibody, *CNI* calcineurin inhibitor, *CsA* cyclosporine, *EVR* everolimus, *MMF* mycophenolate mofetil, *mTOR* mammalian target of rapamycin, *rATG* rabbit antithymocyte globulin, *SIR* sirolimus, *TAC* tacrolimus

[a] Patients #2, #4, and #5 received belatacept with everolimus (CNI therapy was discontinued) owing to non-adherence to CNI therapy

Graft function

Median eGFR declined from 100 (range 57–126) mL/min/1.73 m^2 at 9 months before the cAMR diagnosis to 48 mL/min/1.73 m^2 at the time of diagnosis, with a subsequent decrease to 40 (range 17–57) mL/min/1.73 m^2 at the point of rATG introduction (Fig. 1). Over the 9 months following rATG initiation, it increased progressively to 62 (range 13–87) mL/min/1.73 m^2 ($p = 0.039$ versus time of rATG introduction). Four patients (#4, #5 #8, and #9) showed substantial recovery of eGFR (increasing by between 28 and 41 mL/min/1.73 m^2) and 2 patients showed a small improvement (#3 [10 mL/min/1.73 m^2), #6 [7 mL/min/1.73 m^2; Fig. 2). Three patients continued to show deteriorating renal function after rATG introduction (#1: 36 to 30 mL/min/1.73 m^2; #2: 48 to 21 mL/min/1.73 m^2; #7: 27 to 13 mL/min/1.73 m^2). eGFR values in these 3 patients at the time of cAMR diagnosis were not lower than in the other patients (Fig. 1, Table 1).

Table 3 Pathological evidence for chronic active antibody-mediated rejection according to the Banff 2013 criteria [22]

Patient	#1	#2	#3	#4	#5	#6	#7	#8	#9
Morphological evidence for chronic tissue injury									
Transplant glomerulopathy	0	0	3	0	0	0	3	1	0
Peritubular capillary basement membrane multilayering	2	2	0	1	1	3	2	0	2
New-onset arterial intimal fibrosis	2	0	1	1	3	1	1	2	0
Evidence for current/recent antibody interaction with vascular endothelium									
Linear C4d staining in peritubular capillaries	2	3	0	0	3	0	1	2	0
Microvascular inflammation	1	1	2	1	2	2	2	2	1

DSAs donor-specific antibodies, *HLA* human leukocyte antigen

0 absent, *1* mild, *2* moderate, *3* severe

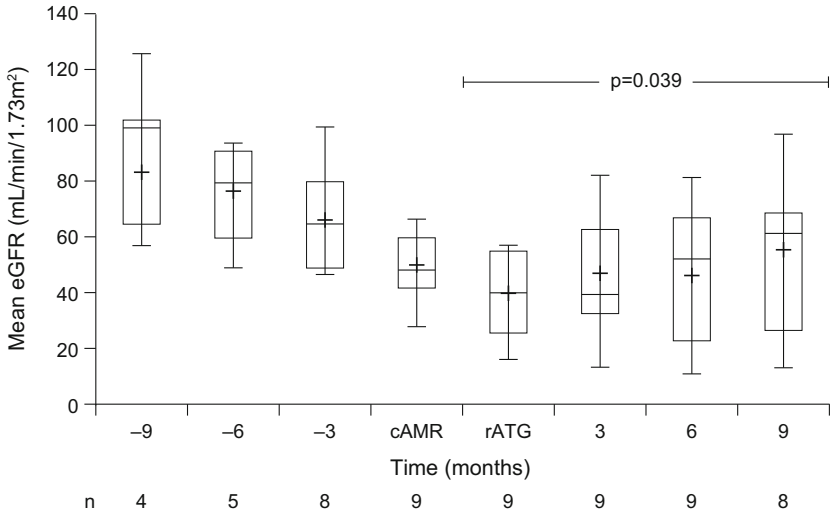

Fig. 1 Estimated glomerular filtration rate (*eGFR*) during the 9 months before the diagnosis of chronic antibody-mediated rejection (*cAMR*) and 9 months after the introduction of rabbit antithymocyte globulin (*rATG*). Data are shown only for post-transplant measurements. *Horizontal bars* indicate median values; *crosses* indicate mean values; *boxes* indicate interquartile ranges; and *whiskers* indicate maximum and minimum values. If only two values were available, individual values are shown

Ratios of urinary albumin/creatinine showed no consistent pattern after the introduction of rATG. At time of cAMR diagnosis, values varied from <5 mg/mmol (patients #1, #4, #5, and #6) to >900 mg/mmol (patients #2 and #3). After the introduction of rATG, the ratio remained stable in 3 patients (#1, #6, and #7), decreased in 3 patients (#3, #5, and #8), and increased in 3 patients (#2, #4, and #9; Fig. 3). The median urinary albumin/creatinine ratio was 22.9 mg/mmol at the time of starting rATG and 13.5 mg/mmol at month 9 ($p = 0.29$).

No grafts were lost during follow-up and none of the patients required dialysis during the 9-month follow-up period after starting rATG.

Tolerability of rATG

No adverse events with a suspected association with rATG administration, including allergic reactions, leukocytopenia or hospitalization for severe infection (or hospitalization for any other reason), were observed in any of the patients. There were no episodes of EBV, cytomegalovirus or BK polyomavirus infections in any of the patients during the observation period.

Discussion

The results presented here suggest that rATG might be a useful component of the armamentarium for treating cAMR. Current management of cAMR remains unsatisfactory, and no drugs are approved for its treatment. The conventional combination of IVIG and rituximab has largely been adopted based on its successful use in desensitizing highly sensitized patients before kidney transplantation [37]. New therapies have been investigated, including induction of plasma cell apoptosis by the proteasome inhibitor bortezomib, but this does not appear to reduce DSA levels when used as a monotherapy [38]. Novel agents, for example, the monoclonal anti-interleukin 6 receptor antibody [39] and C1 esterase inhibitor, have shown early promise [39], and the complement inhibitor eculizumab

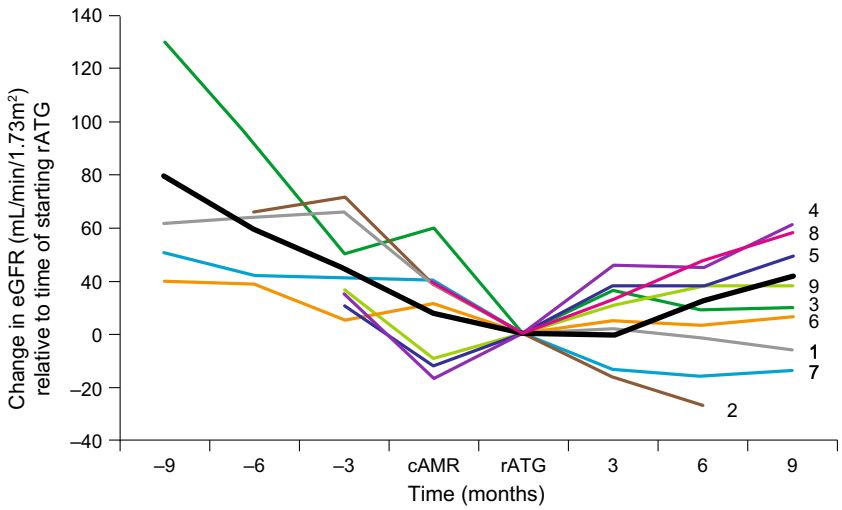

Fig. 2 Individual values for the change in estimated glomerular filtration rate (eGFR) during the 9 months before the diagnosis of chronic antibody-mediated rejection (cAMR) and 9 months after the introduction of rabbit anti-human thymocyte globulin (rATG), with eGFR at the time of starting rATG as the reference point (zero). Median values are shown in *black*

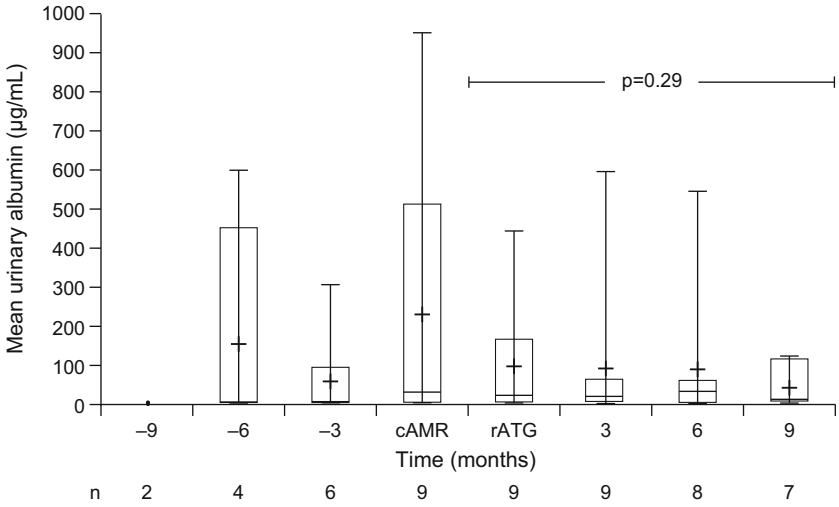

Fig. 3 Urinary albumin/creatinine ratio during the 9 months before the diagnosis of chronic antibody-mediated rejection (cAMR) and 9 months after the introduction of rabbit anti-human thymocyte globulin (rATG). Data are shown only for post-transplant measurements. *Horizontal bars* indicate median values; *crosses* indicate mean values; *boxes* indicate interquartile ranges; and *whiskers* indicate maximum and minimum values. If only two values were available, individual values are shown

[40] may potentially be helpful in patients with intensive activation of the terminal complement complex, but firm evidence is not yet available. Against this background, application of rATG—an agent familiar to the transplant community over many years of use—is of potential interest.

In this series of nine children with cAMR and declining renal function, the addition of a 5-day course of rATG as a rescue therapy was associated with a substantial improvement in graft function in 4 cases and a small improvement in a further 2 cases. The remaining 3 patients continued to deteriorate. Strikingly, the 4 patients in whom eGFR improved (#4, #5, #8, and #9) all had cAMR diagnosed within the first 4 months after transplantation; late-onset cAMR proved less responsive. In 4 of the 8 patients in whom post-transplant Luminex data were available, DSAs present at the time of cAMR diagnosis had become undetectable, and MFI values were reduced after the intensive combined immunosuppressive treatment in 1 further patient. The remaining 3 patients still had significant levels of anti-HLA-DQ DSA, which is often the dominant form of DSAs [41] and is frequently hard to eliminate. No consistent changes were detected concerning the ratio of urinary albumin to creatinine.

The key safety concerns related to rATG therapy—higher risk for malignancy or infection—have diminished substantially under modern dosing regimens [42, 43]. In our series, no leukocytopenia or thrombocytopenia was detected. Despite the intensive immunosuppressive regimens administered, none of the patients had to be hospitalized because of severe infections. rATG infusions were generally well-tolerated in our patients and the complete rATG course of five injections was administered as planned in all 9 children. Nevertheless, longer-term follow-up would be essential in future studies of rATG for the management of cAMR in children to monitor risks, particularly for post-transplant lymphoproliferative disorder.

This cohort represented a hard-to-treat group in whom a series of other interventions had failed to arrest the decline in graft function. Thus, although numbers are small, these results are highly encouraging. We did not initiate rATG earlier as the evidence base is inadequate. Future studies could usefully explore the earlier introduction of rATG, likely in combination with one or more other therapies such as rituximab. The 3 patients who continued to deteriorate despite rATG therapy, showed no distinguishing features compared with responders, although none had been diagnosed with cAMR early post-transplant. A longer-term goal would be to determine which cases of cAMR are most likely to respond to rATG therapy.

Immunologically, the addition of rATG to rituximab appears rational. rATG includes a wide range of T-cell and non-T-cell antigen specificities, including antibodies against B-cell, plasma cell, and natural killer (NK) cell markers [44, 45], and in vitro it induces complement-independent apoptosis of activation and naïve B-cells, and plasma cells [46]. Clinically, T-cells and NK cells are depleted under rATG administration [19–21]. NK cells are central to the inflammatory processes in the graft during AMR and their depletion may promote graft survival [47]. Mature B-cells and plasma cells remain unaffected by rATG in vivo [19–21], but $CD19^{+}CD27^{+}$ memory and switch memory B-cells appear to be selectively suppressed for a prolonged period when rATG is used in desensitization protocols or as induction before kidney transplantation [19–23]. It seems feasible that rATG might inhibit memory B-cells by suppressing the availability of antigen-specific helper T-cells. Rituximab, in contrast, depletes mature B-cells [21, 46], but is less effective at depleting memory B-cells [21, 48, 49]. The immunological effects of the two agents may thus be complementary for targeting B-cells and their antibodies in patients with AMR.

Published data relating to rATG treatment of AMR are remarkably sparse and to our knowledge limited to cases of

acute AMR, not cAMR. In one early study, published in 2004, a total of 27 patients with presumed acute AMR were treated with rituximab and plasmapheresis, with steroids in most cases, and 22 of the patients also received rATG [49]. Outcomes were good, with a death-censored graft survival rate of 89% after a mean of 605 days' follow-up [50]. Zheng et al. treated 4 adults with acute AMR using rATG monotherapy and concluded that mild or moderate AMR was ameliorated, but not cured [51]. Two other published case reports have described successful outcomes using rATG combined with eculizumab, plasmapheresis, and IVIG [52] or high-dose corticosteroids, IVIG, and plasmapheresis [53].

We recognize that this represents a small study population. This retrospective analysis has additional limitations. The group of patients was notably heterogeneous. There were wide variations in the time between diagnosis of cAMR and initiation of rATG, and the individualized treatment regimens prescribed before rATG therapy also differed between patients, for example, with or without the introduction of belatacept and/or bortezomib therapy, although all patients were treated with steroid pulses, rituximab, and intravenous immunoglobulins. Inevitably, this limits the strength of conclusions that can be drawn about the effects of rATG as a single agent. Bortezomib has been associated with a transient decrease in DSA levels when used to treat AMR in pediatric kidney transplant recipients [54, 55], which may have contributed to outcomes. Additionally, pre-implantation and surveillance biopsies, in addition to biopsies and measurement of DSA MFIs directly before and after rATG administration, would have provided useful information, but were not performed because of the retrospective nature of this study. Similarly, monitoring of T-cell and B-cell subsets would have been helpful. However, this small cohort is relatively typical of pediatric kidney transplant patients, including the high incidence of non-adherence, necessitating a switch in immunosuppression, which is a leading risk factor for the development of DSAs [56]. There was also extensive HLA mismatching (from 2 to 8 mismatches) in the group, characteristic of the problems of achieving good matches in pediatric recipients.

Nevertheless, the current series of patients represents the largest dataset so far regarding use of rATG to treat cAMR. Used here as rescue therapy after a poor response to a sequence of other interventions, rATG appears a promising component of the treatment regimen. In the absence of prospective or comparative analysis, these observations may help to inform treatment choices in this challenging situation, and suggest that rATG might be a viable option for managing cAMR. However, an important caveat is that all patients had previously received multiple interventions in response to the diagnosis of cAMR, and it is not possible to reliably disentangle the effect of rATG. Prospective trials are urgently required, designed specifically to examine the impact of rATG, with adequate follow-up for safety monitoring. Investigation into the earlier introduction of rATG into the management of cAMR should also be carried out.

Compliance with ethical standards

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Ethics This retrospective study did not require ethical approval.

Conflicts of interest Lars Pape has received speaker's honoraria and travel grants from Novartis Pharmaceuticals. None of the other authors has any conflicts of interest to declare.

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