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Clearance of C4d deposition after successful treatment of acute humoral rejection in follow-up biopsies: a report of three cases

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Abstract Acute humoral rejection (AHR) is currently perceived as an immunological reaction against donor antigens mediated by complement-binding antibodies. C4d, a split product of complement activation and bound to endothelial cells of the peritubular capillaries, is used as a diagnostic marker for AHR. We report on three patients with biopsy-proven acute humoral rejection who were treated initially with plasmapheresis (PS). As two of the patients did not recover renal function, and biopsy showed persistent C4d staining after PS, immunoadsorption (IAS) was additionally performed on them. In all patients, renal function recovered, and follow-up biopsies in two patients showed complete disappearance of C4d, 29 days and 58 days after transplantation and only minimal residual C4d deposits in one patient 48 days after transplantation. We conclude that successful treatment of AHR is followed by complete resolution of serological and histological markers of AHR, displayed by the disappearance of C4d.

Keywords Transplantation · C4d · Acute rejection · Humoral rejection · Plasmapheresis · Immunoadsorption

Introduction

The need for differentiation of acute rejection (AR) into acute cellular rejection (ACR) and acute humoral rejection (AHR) is becoming increasingly important because of different therapeutic options. Only recently, AHR has been accepted as its own category in the Banff classification system, and is characterised by the triad of serological (donor-specific antibodies), histological (neutrophils in peritubular capillaries; arterial fibrinoid necrosis; acute tubular injury) and immunological (deposition of C4d) markers in severe early graft dysfunction resistant to conventional (steroid) anti-rejection therapy [1]. Until recently, AHR has been associated with poor graft survival. However, successful treatment, with recovery of graft function, has been described with various strategies, including plasmapheresis (PS) combined with tacrolimus–mycophenolate mofetil rescue therapy, intravenous immune globulin and immunoadsorption (IAS) [2, 3, 4, 5, 6, 7, 8].

The role of C4d deposits in peritubular capillaries (PTCs) in AHR has been studied extensively in recent years. In the search of significant immunohistological criteria for AHR, C4d deposition has been shown to be a sensitive, specific and important prognostic factor [9, 10, 11]. Furthermore, the presence of C4d might be the only evidence of AHR [12]. Detection of immunoglobulins and complement split products during humoral attack has been shown to depend on the rapidity of their turnover [13, 14]. Clearance of antibodies, antigens and complement components is thought to be mediated by internalisation or shedding from the endothelial cell membrane. In contrast to other complement factors, C4d binds, after cleavage through factor I,

covalently to structures surrounding the PTC endothelium, escaping, thereby, early removal from the target organ. C4d deposits persist throughout a humoral attack and are, therefore, viewed as a footprint of humoral rejection. Whether successful therapy of AHR and cessation of antibody generation are accompanied by alterations of immunological (C4d) and serological markers of AHR is unclear. We report on three patients with successful reversal of AHR after intensive antihumoral therapy, and showed complete disappearance of C4d in follow-up biopsies.

Case reports

Patient 1

Patient 1 was a 37-year-old man with end-stage renal disease, due to hereditary adenine phosphoribosyltransferase deficiency, and was receiving his fourth cadaveric renal transplant, as former grafts had been lost to chronic rejection. Baseline immunosuppression (IS) consisted of tacrolimus, steroids and rapamycin. Latest lymphocytotoxic antibody panel reactivity (PRA) value before transplantation was 45%, and lymphocytotoxic crossmatch was negative. Because of initial graft failure, the patient was subjected to intermittent dialysis (Fig. 1). After exclusion of haemodynamic and post-renal causes of initial graft failure and calcineurin-inhibitor toxicity, the patient underwent a kidney biopsy on postoperative day (pod) 7. Histology showed glomerulitis with microthrombi in the glomerular capillaries and granulocyte infiltration of peritubular arterioles, as well as peritubular capillary C4d deposition. PRA level on pod 10 was 92%. There was no evidence of ACR. Antirejection therapy was started with a steroid bolus and six courses of plasmapheresis (PS) (19,534 ml treated plasma volume) on pods 7–15. Graft function did not recover, and lymphocytotoxic crossmatch was positive on pod 15. On pod 17 the patient underwent repeated biopsy, which showed unchanged C4d deposits with decreased signs of granulocyte infiltration. Persistent humoral rejection was suspected, and 16 sessions of IAS were initiated (89,833 ml treated plasma volume, pods 21–58). The patient was subjected to dialysis intermittently during the whole course of treatment. On

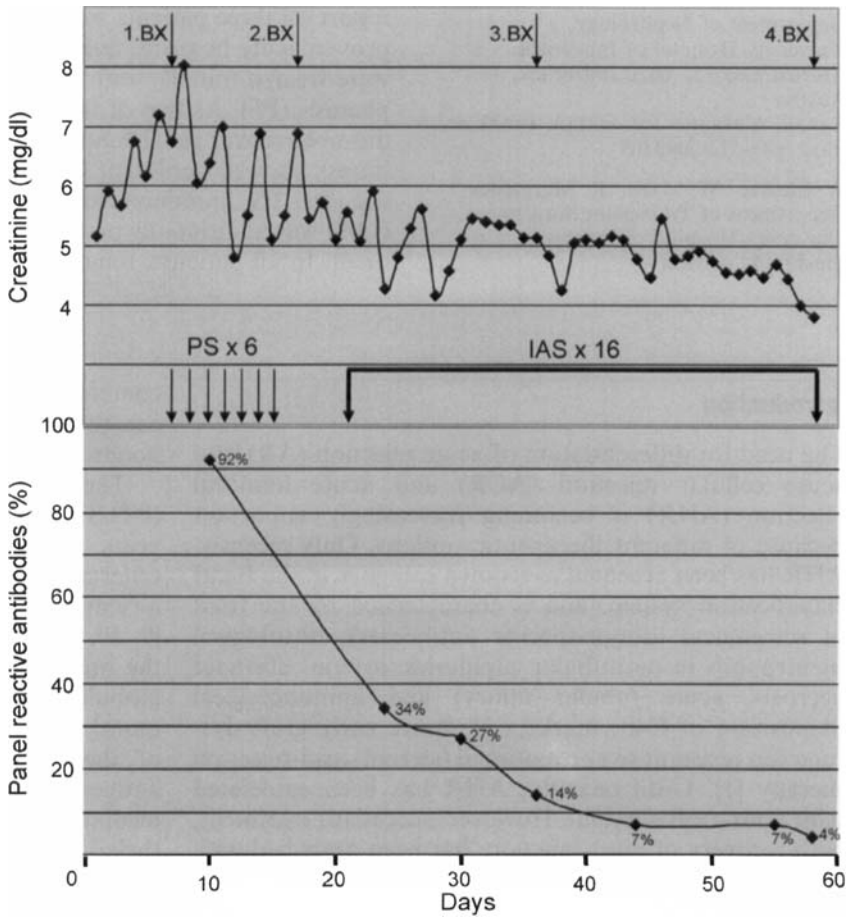

Fig. 1 Clinical course and therapeutic interventions in patient 1 (*BX* biopsy, *IAS* immunoadsorption, *PS* plasmapheresis)

pod 36, follow-up biopsy due to ongoing graft dysfunction revealed discrete focal interstitial lymphocytic infiltrate, with no signs of acute rejection and only trace C4d deposits. Intermittent haemodialysis and IAS were continued. On pod 58 a follow-up biopsy showed complete disappearance of C4d. PRA levels at that time were 4%, and IAS treatment was stopped. Additionally, allopurinol was started due to the patient's adenine phosphoribosyltransferase deficiency. In the following days, a marked improvement of renal function was noted, and haemodialysis was discontinued.

Patient 2

This patient was a 52-year-old man with end-stage renal disease (ESRD) of unknown aetiology, who had received his second renal transplant after loss to chronic rejection. Baseline immunosuppression consisted of cyclosporine, steroids and azathioprine. Latest lymphocytotoxic antibody PRA before transplantation was 32% (highest measured PRA 42%), and lymphocytotoxic crossmatch was negative. After initial urine output on pod 1, graft function decreased on the following day, and azathioprine was switched to mycophenolate mofetil on pod 2. After exclusion of haemodynamic and post-renal causes of initial graft dysfunction, dialysis for calcineurin-inhibitor toxicity was initiated, and the patient received a steroid bolus on pod 4-6. On pod 5, the patient underwent a kidney biopsy, which showed glomerular infiltration with granulocytes, but no evidence of ACR. C4d staining and PRA measurement were not performed, since humoral rejection was not suspected. Decreased diastolic Doppler flow signal of the renal artery was detected on pod 8, and treatment was switched from cyclosporine to tacrolimus. Due to persistent graft dysfunction, administration of a steroid bolus was repeated (pods 11–12), and a second graft biopsy was performed on pod 11. Interstitial and glomerular granulocyte infiltration was observed in the biopsy specimen, together with signs of ACR, classified as Banff IIa. C4d staining of PTCs was markedly positive, together with the presence of PRA. Anti-rejection treatment was started with antithymocyte globulin (ATG-Fresenius) and six courses of PS (exchanged plasma volume 18,522 ml, pods 12–20). Renal function significantly improved on pod 18, and dialysis was discontinued. On pod 33 a brisk increase in serum creatinine level and a decrease in urine production were noted. Graft biopsy was performed, and histology showed ACR (Banff IIa), with C4d deposits in PTCs. PRA levels at that time were 42%. A steroid bolus was administered (pods 34–36), rapamycin was added to baseline IS, and IAS (nine courses, treated plasma volume 53,646 ml, pods 35–48) was started, due to suspected humoral rejection on pod 35. In the following days graft function improved markedly. Follow-up biopsy on pod 48 revealed only trace evidence of C4d, with no detectable PRA levels, and IAS was discontinued.

Patient 3

Patient 3 was a 36-year-old woman with ESRD due to focal glomerulosclerosis, and she had received her first renal transplant. Baseline IS comprised cyclosporine, steroids and azathioprine. Lymphocytotoxic antibody PRA and lymphocytotoxic crossmatch were negative before transplantation. After initial graft function, urine production ceased on pod 4. After exclusion of haemodynamic and post-renal causes of initial graft failure and calcineurin-inhibitor toxicity, steroids were administered, and the baseline IS was changed from azathioprine to mycophenolate mofetil on pod 5. Graft dysfunction persisted and dialysis was initiated. On pod 8, a transplant biopsy was performed that showed Banff IIa ACR, with abundant granulocytes in the interstitial space, together with fibrinoid necrosis of arterioles and focal endotheliitis. PTCs showed prominent C4d deposits. Six courses of PS (exchanged plasma volume 21,003 ml, pods 8–17) and antithymocyte therapy (ATG-Fresenius) were administered, and cyclosporine was replaced by tacrolimus. Graft function recovered slowly, and dialysis was discontinued on pod 18. PRA measurement on pod 15 was negative. Because of poor graft function, kidney biopsy was repeated on pod 29 and showed no evidence of rejection and absence of C4d deposits. The dose of tacrolimus was reduced, rapamycin was added, and graft function improved in the following days.

Materials and methods

Histological diagnosis and grading of allograft rejection was performed according to the criteria of the Banff 97 Working Classification of Renal Allograft Pathology [15]. Immunofluorescence staining with anti-human C4d monoclonal antibody (Quidel, Heidelberg, Germany) was performed on a snap-frozen portion of the allograft biopsy. Intensity of endothelial C4d staining was staged according to Böhmig et al. [3].

Plasma was separated with a traditional plasma separator, passed through the column for adsorption, and then re-infused. For antibody-based IgG-immunoadsorption two columns containing 150 ml of Sepharose-coupled polyclonal sheep antibodies to human immunoglobulin (IgG, IgA, IgM), heavy and light chains (Ig-Therasorb, Plasmaselect, Teterow, Germany) were used. Each column had an immunoglobulin binding capacity of approximately 0.8 g–1.2 g per cycle.

Discussion

Two of our patients had several risk factors for AHR, and severe dialysis-dependent graft dysfunction developed in all patients in the early post-transplantation period (Table 1). According to the newly revised Banff criteria by Racusen et al., graft biopsies in patient 1 were classified as antibody-mediated rejection, by fulfilling all suggested criteria, and as "suspicious for AHR" in patients 2 and 3 (Table 2) [1]. Initial treatment consisted of steroid bolus therapy and PS, and additional IAS therapy in two patients due to persistent or recurrent graft dysfunction. In all our patients severe dialysis-dependent graft dysfunction was reversed (Table 3), and C4d had disappeared in the follow-up biopsies. To our knowledge, this is the first time that disappearance of C4d has been observed after successful therapy of humoral rejection.

AHR is currently perceived as an immunological reaction against donor antigens, mediated by complement-binding antibodies and followed by the activation of the complement system. C4d, a split product of C4, is derived from the classical pathway of the complement system after cleavage through factor I. During ongoing graft injury, C4d is deposited in the peritubular capillaries (PTCs). Compared with other components of humoral rejection, such as IgG or C3, C4d has been shown to be a more durable marker of AHR by binding covalently to PTCs [9, 16]. After cessation of graft injury, C4d is cleared from the tissue by currently unknown pathways. Consistent with this hypothesis, we observed a remarkable association between levels of PRA and the intensity of C4d deposition in our patients. High levels of PRA correlated, always, with prominent C4d staining in our patients, while a trend to weaker staining intensity was noted with decreased PRA levels. Time of disappearance of C4d after initial biopsy was variable, ranging from 21 days (patient 3) to 37 days and 51 days (patients 1 and 2). These events, however, were preceded by an earlier recovery of graft function, indicating that the presence of C4d does not compromise graft function, and confirming its role as an innocent by-product of a complex immunological reaction culminating in AHR.

Successful treatment of AHR with PS and IAS has been previously shown to affect histological and serological markers of AHR. Böhmig et al. reported histological (decreased granulocyte accumulation; glomerulitis; intimal arteritis) and serological (decreased PRA reactivity) changes associated with AHR in follow up of patients after successful treatment with IAS [3]. Disappearance of C4d, however, was not observed, and non-responders did not show any of the features mentioned above. Negative crossmatches and negative leukocyte antibody screens after PS and IAS have also been reported by others [17].

The strategy of depletion of the probable causative agent (anti-donor antibodies) of AHR by PS or IAS was first used in highly sensitised patients with

Table 2 Diagnosis of acute humoral rejection

Parameter	Patient 1	Patient 2	Patient 3
C4d in PTCs	Yes	Yes	Yes
Neutrophils in			
Glomeruli	Yes	Yes	Yes
PTCs	Yes	No	Yes
Tubules	No	No	Yes
Fibrin thrombi			
Glomeruli	Yes	No	Yes
Fibrinoid necrosis			
Arteries	Yes	No	Yes
Donor-specific antibodies	Yes	Yes	No

Table 3 Outcome of graft function

Patient no.	Reversal of AHR	Creatine at time of AHR	Creatine after PS/IAS (pod)	Current creatine (Months)
1	Yes	HD[a]	4.9 mg/dl (61)	2.4 mg/dl (9)
2	Yes	HD[a]	2.1 mg/dl (48)	1.6 mg/dl (8)
3	Yes	HD[a]	2.9 mg/dl (31)	2.4 mg/dl (8)

[a]Haemodialysis dependent

Table 1 Patients' characteristics (*NA* not applicable, *m* male, *f* female)

Characteristic	Patient 1	Patient 2	Patient 3
Age (years)	36	53	36
Gender	M	M	F
Number of previous transplants	3	1	0
Number of previous pregnancies	NA	NA	2
Prior blood transfusions	No	No	Yes
Last PRA before transplantation (%)	45	32	0
Lymphocytotoxic crossmatch	Negative	Negative	Negative before transplantation
Number of HLA mismatches	3	4	3
Delayed graft function	Yes	Yes	No
Time of diagnosis of AHR (days)	7	11	8

increased PRA levels prior to transplantation, in order that AHR be prevented [18]. Later, these treatment modalities have also been shown to reverse AHR once it has occurred [6, 7, 8]. In all patients PS was started because of humoral rejection, and if this was not effective (patients 1 and 2), we switched to IAS. Pretransplantation IAS or PS was not performed in our patients and is only performed at our centre if PRA levels are higher than 70%. The treatment period of specific anti-humoral therapy (PS and IAS) varied considerably in our patients, ranging from 10 days (patient 3) to 21 days (patient 2) and 48 days (patient 1). The duration of treatment was paralleled by decreasing PRA levels (Fig. 1), which might be viewed as a surrogate of treatment efficacy. While there is no evidence of superiority of one treatment modality over the other, IAS has the theoretical advantage of higher specificity in removing antibodies, thereby sparing the patient's valuable plasma proteins during long-term therapy and ensuring less danger of transmission of infections such as hepatitis C. However, co-existence of ACR in two patients (patients 2 and 3) required the addition of anti-T lymphocyte therapy, a therapy that has also been reported to affect AHR [19]. Therefore, a beneficial effect of this therapy cannot be ruled out.

In summary, our report provides further information about the relationship of anti-humoral therapy, graft function recovery and disappearance of markers of AHR. Our experience supports the hypothesis that successful treatment of AHR, by removal of the causative agent (anti-donor antibodies), is associated with complete resolution of serological (PRA) and histological markers of AHR, the latter displayed by the disappearance of C4d.

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