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Treatment of early mixed cellular and humoral renal allograft rejection with tacrolimus and mycophenolate mofetil

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This prospective study investigated the efficiency of the tacrolimus (Tac) combined with mycophenolate mofetil (MMF) alone without immunoadsorption (IA) or plasmapheresis (PPH) as treatment for early (within 2 weeks) acute humoral rejection (AHR) in non-sensitized renal allograft recipients. Of 160 patients enrolled in this prospective study, 11 patients had histologically and clinically confirmed early steroid-resistant acute rejection with an antibody response and received Tac-MMF therapy. No other aggressive rescue methods such as IA, PPH were used, according to the study design. Patients ($n = 11$) were followed for 13.8 ± 3.5months; nine were females. The complement-dependent cytotoxicity crossmatch was negative before transplantation in all patients and only positive for panel-reactive antibody in one patient. Most of the rejection episodes were mixed with cellular rejection (four patients met Banff IIA criteria, five patients met Banff IIB, one patient met Banff IB, and one patient met Banff borderline). After 16.19 ± 6.16 days of treatment, all rejection episodes were successfully reversed and all graft functions were stable, with a mean serum creatinine level of 1.12 ± 0.32 mg/dl during follow-up. No patient suffered from severe infectious complications (except one case of urinary infection). Our investigation suggests that Tac combined with MMF alone is adequate to reverse early mixed cellular and humoral C4d-positive rejection in non-sensitized renal allograft recipients.

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Alloantibody-mediated acute rejection (AR), also called acute humoral rejection (AHR), constitutes 20–30% of AR cases in kidney transplant recipients, and is associated with a poor prognosis.[1,2] It manifests as severe, mostly steroid-resistant, graft dysfunction, and is associated with a high rate of graft loss.[3–5] In order to reduce the incidence of AHR, the screening of patients for pre-existing donor-specific antibodies (DSAs) was strengthened. However, DSAs, especially human lymphocyte antigen-II-reactive antibodies, are not detectable by conventional methods, whereas very low levels can be associated with the occurrence of severe AHR post transplant.[6]

Combined use of immunoadsorption (IA) or plasmapheresis (PPH), mycophenolate mofetil (MMF) (CellCept®), tacrolimus (Tac), and/or intravenous immunoglobulins (IVIG) has been reported to effectively reverse AHR.[6–9] Unfortunately, these strategies are costly and are often complicated by patients developing severe infections.[10–12] The treatment is prohibitively expensive for many patients, particularly those in developing countries. However, some studies have suggested that IA or PPH may not be necessary in all AHR patients. Nickeleit *et al.*[13] reported that antilymphocytic preparation without IA or PPH can effectively rescue C4d-positive rejection in patients with pronounced allograft dysfunction; IA or PP has not been used in their cohort too. Koo *et al.*[14] also observed a good outcome with anti-thymocyte globulin treatment of early AHR without IA or PPH.

On the other hand, we found in our previous studies[12] that outcome of graft is significantly associated with the onset time of AHR. AHR occurring during the first 2 weeks had a statistically better outcome than that occurring more than 2 weeks after transplantation. A further study[15] revealed that AHR onset at different time points is associated with unique clinico-histopathological manifestations, and suggested that early and late AHR are each associated with a different pathogenesis, and may need to be treated separately. Thus, we began to wonder whether IA or PPH and other aggressive strategies could be dispensable for patients with early AHR.

Tac combined with MMF (Tac-MMF) has been used effectively in the treatment of steroid-resistant AR for many years.[16] In a recent study,[12] we retrospectively stained C4d in

grafts with steroid-resistant AR, and were surprised to find that >80% of Chinese recipients with C4d-positive steroid-resistant AR could be treated by the simple combination of Tac-MMF without the need for IA or PPH. We believe most of these episodes of AR included an antibody-dependent component, in addition to the cellular rejection. This finding concurred with the result of a previous study reported by Pascual *et al.*,[17] in which, although they used PPH concomitantly, they noticed that Tac-MMF therapy was critical for successful reversal of AHR.

Based on these findings, it seems that Tac-MMF might be adequate to treat early AHR after transplantation. This prospective study aims to further investigate the effectiveness of a combination of Tac-MMF alone as a treatment of early (within 2 weeks) AR with an antibody response in Chinese renal allograft recipients.

RESULTS

Baseline patient characteristics

One hundred and sixty patients were enrolled in this study, and 20 episodes of AR occurred during the first 2 weeks, all episodes were proved by biopsy and stained for C4d. Thirteen were C4d-positive and of these, 11 out of 160 patients (7%) were diagnosed as having early steroid-resistant AR with an antibody response (AHR) during the 14-month investigation. The demographics and the underlying risk factors of the 11 recipients are listed in Table 1. The median age of patients was 39 years. The proportion of females experiencing early AHR was significantly greater than the proportion of males (13.8% (9/65) versus 2.1% (2/95), $P<0.01$). All of the nine females had previously been pregnant (range: 1–4 previous pregnancies). All patients were receiving their first renal allograft. All transplantations were ABO blood group-compatible and pre-transplant complement-dependent cytotoxicity (CDC) crossmatch were negative. Only one patient (patient 6) had a positive panel-reactive antibody (PRA) before transplantation (patients with positive PRA and/or CDC crossmatch are not usually considered for renal transplantation in our center). Six recipients received primary immunosuppression consisting of cyclosporine A (CsA), MMF, and steroids, and two of these patients also received two doses of basiliximab as induction therapy. The other five patients were treated with Tac (initial dose: 1.25 mg/kg day^{-1}), MMF, and steroids, and among these patients two had received two doses of daclizumab as induction, whereas one had received two doses of basiliximab as induction. The use of interleukin-2 monoclonal antibody as induction therapy had no effect on the incidence of AHR (7 versus 6% for with versus without interleukin-2 antibody, respectively; $P=0.839$), even though its use significantly decreased acute cellular rejection (3 versus 12%; $P=0.039$).

Clinical manifestations

All of the AHR episodes occurred in the first week (median 4 days post transplant (range: 1–7 days)). The majority of recipients ($n=10$) experienced an improvement in initial graft function (reflected by decrease of serum creatinine levels) before the AHR occurred (Table 2). Fever, decrease of urine volume, and progressive allograft dysfunction were observed as onset manifestation of AHR in all the 11 recipients. Ultrasound examination revealed an enlarged renal allograft and increased vessel resistance index in each recipient. Delayed graft function, defined as a need for renal replacement therapy within the first week of transplant, occurred in six recipients. No malignant hypertension was present in this cohort.

Pathology findings

Diagnostic biopsies were performed after the onset of rejection (median 4 days post onset of rejection (range: 2–11 days)). All patients had neutrophil infiltration in peritubular capillaries (PTCs), and glomerulitis with neutrophils or mononuclear cells infiltration was found in nine (82%) of 11 diagnostic biopsies (Table 3). Tubulitis was observed in 36% patients. Three patients had interstitial hemorrhage. Endo-arteritis was found in all but two cases. Most episodes of rejection were mixed with cellular rejection. According to Banff 97 criteria, four patients met the criteria of IIA, five patients met the criteria of IIB, one patient met

Table 1 | Patients demographics

Case	Sex/age	Underlying renal disease	Previous pregnancies	Pre-transplant perfusion	Previous transplant	Cold ischemia time (h)
1	F/54	Interstitial nephritis	4	No	No	8.5
2	F/36	CGN	3	No	No	16.3
3	F/39	CGN	1	No	No	19.5
4	F/39	Polycystic kidney	3	No	No	6.7
5	F/42	CGN	1	No	No	12.0
6	F/42	CGN	1	No	No	9.3
7	F/43	MsPGN	4	No	No	10.5
8	M/30	CGN	—	No	No	10.2
9	F/39	CGN	2	No	No	13.5
10	M/30	FSGS	—	No	No	8.8
11	F/42	IgAN, FSGS	3	No	No	11.3

CGN, chronic glomerularnephritis; F, female; FSGS, focal segmental glomerulosclerosis, IgAN, IgA nephritis; M, male; MsPGN, mesangioproliferative glomerulonephritis.

Table 2 | Clinical characteristics

Case	Onset of AR[a] (days)	Diagnostic biopsy[b] (days)	Initial therapy	Dose/trough levels of CNIs (mg kg^{-1} day^{-1}/ng ml^{-1}) Initial	Rescue	Diagnosis of rejection Scr (mg/dl)	Urine output (ml/day)	Time of effect[b] (days)	Reversal of rejection	Current Scr (mg/dl, months)
1	7	2	D+T+M+P	0.10/10.9	0.10/10.0	4.17	250	20	Yes	0.88 (14)
2	2	4	C+M+P	5.5/208	0.13/12.5	CVVH	760	22	Yes	1.00 (13)
3	4	2	C+M+P	5.4/186	0.14/14.5	6.97	640	20	Yes	1.56 (21)
4	1	6	D+T+M+P	0.12/(Not test)	0.12/10.4	6.36	920	20	Yes	0.94 (13)
5	4	6	B+C+M+P	5.1/160	0.15/7.1	CVVH	895	14	Yes	0.91 (13)
6	3	7	B+C+M+P	5.1/102.6	0.12/10.9	CVVH	330	16	Yes	0.96 (11)
7	4	2	C+M+P	5.0/176	0.13/9.1	4.79	1280	12	Yes	1.37 (11)
8	6	2	B+T+M+P	0.12/12.8	0.12/11.2	9.04	1100	12	Yes	1.74 (10)
9	4	4	C+M+P	6.1/348	0.12/12.0	3.26	1050	4	Yes	0.88 (11)
10	2	6	T+M+P	0.11/5.7	0.13/9.2	CVVH	1915	12	Yes	1.24 (16)
11	3	2	T+M+P	0.10/8.3	0.11/9.5	CVVH	150	26	Yes	0.79 (19)

B, basiliximab; CNI, calcineurin inhibitor; CVVH, continuous veno-venous hemofiltration; D, daclizumab; M, mycophenolate mofetil; P, prednisolone; Scr, serum creatinine; T, tacrolimus.
[a]Days post-transplantation.
[b]Days post onset of rejection.

Table 3 | Immunohistochemical and histological evaluation

Case	C4d	Inflammatory cell infiltration PTC	Glomeruli	Interstitial	Interstitial hemorrhage	Endarteritis	Tubulitis	Banff97 grade
1	+	+	–	–	–	+	–	IIA
2	+	+++	++	+	–	+++	–	IIB
3	+	++	+	+	–	+++	–	IIB
4	+	+	++	–	–	+	+	IIA
5	+	++	+++	–	+	+++	–	IIB
6	+	+++	+++	–	+	++	+	IIA
7	+	+	++	–	–	++	–	IIA
8	+	++	+++	–	+	+++	–	IIB
9	+	+	++	–	–	–	–	Borderline
10	+	++	–	+	–	–	+	IB
11	+	+++	++	+	–	+++	+	IIB

PTC, peritubular capillary.
+, ++, +++=cortical PTC with 3–4, 5–10, >10 luminal inflammatory cells infiltration, glomeruli with 3–4, 5–10, >10 inflammatory cells infiltration; endarteritis meet the Banff criteria v1, v2, v3.

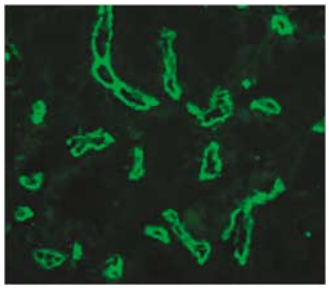

Figure 1 | C4d staining in peri-tubular area in renal allograft with acute humoral rejection. Original magnification × 400.

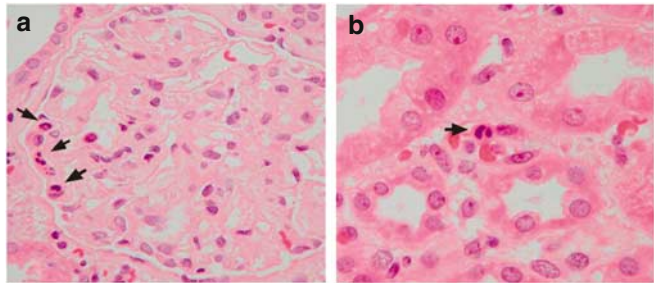

Figure 2 | Typical histological lesions in renal allograft with acute humoral rejection, (a) neutrophils infiltration in glomeruli, (b) neutrophils infiltration in peri-tubular area. Original magnification × 400.

IB, and one patient met borderline. C4d staining was positive in frozen slides in all the 11 patients (Table 3 and Figures 1 and 2). A positive C4d staining was defined as bright linear staining along capillary basement membranes, involving over half of sampled capillaries according to 2001 and 2003 Banff Meetings.[18,19] In addition to C4d staining of the PTCs, the majority of patients also exhibited C3 staining of the interstitial vasculature which was colocalized with C1q and IgM in four patients.

Detection of DSAs

In order to determine the level of DSAs after transplantation, cytotoxic crossmatches and PRA were performed on the day

Table 4 | Variations of panel reactivity antibody and cytotoxic crossmatch on diagnosis

Case	Panel reactivity antibody (HLA-I/II%)		CDC crossmatch	
	Pre-transplant	On diagnosis	Pre-transplant	On diagnosis
1	1.26/4.28	12.0/0.87	Negative	Positive
2	0.75/1.23	2.22/18.1	Negative	NA
3	5.62/2.33	4.82/3.96	Negative	NA
4	0.24/1.98	1.47/0.55	Negative	NA
5	0.14/0.08	16.7/15.6	Negative	Positive
6	3.65/40	18.8/1.39	Negative	NA
7	2.65/0.08	1.78/50.0	Negative	Positive
8	0.74/3.66	2.88/0.57	Negative	Positive
9	0.10/3.57	2.73/44.1	Negative	Positive
10	0.47/1.22	21.2/1.0	Negative	Positive
11	2.03/2.14	46.0/22.5	Negative	Positive

CDC, complement-dependent cytotoxicity; HLA, human lymphocyte antigen. NA, not available, donor lymphocytes were not available in these four patients.

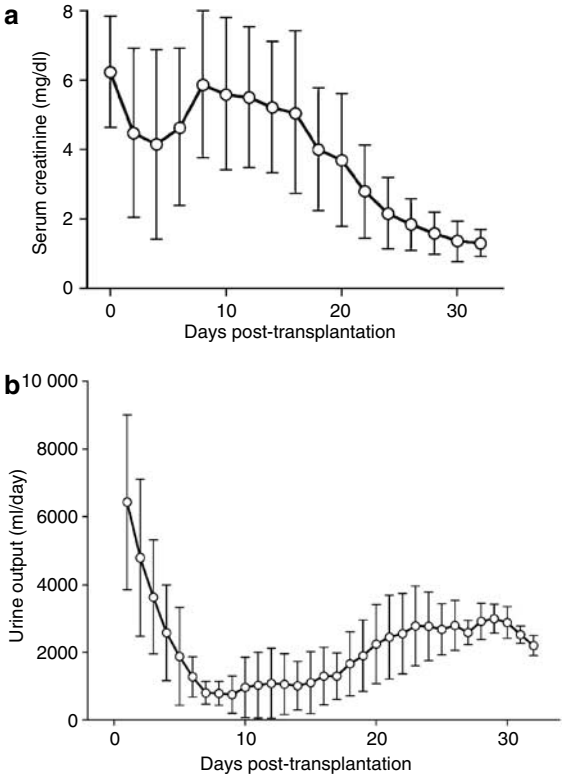

Figure 3 | Recovery of graft function during the course of treatment. It is reflected through (**a**) reduction in serum creatinine and (**b**) increase in urine output. Data are presented as mean ± standard deviation.

of diagnosis. Eight patients were positive for PRA (sera with >10% flow-PRA class I and/or II reactivity). Donor lymphocytes were available in seven patients, and CDC crossmatches were all positive (Table 4). In total, nine patients had evidence of DSAs and could be diagnosed as AHR. Two other patients could be diagnosed with suspicious AHR according to published criteria.[20]

Rescue treatment and clinical outcome

Following detection of AHR, anti-rejection therapy consisting of steroid bolus (methylprednisolone 500 mg for 3 days) and a combination of Tac and MMF was used. No patient had an immediate response to steroid treatment and graft function deteriorated as manifested by decreased urine volume and increased serum creatinine; 10 patients received continuous renal replacement therapy during the treatment course. After 16.2 ± 6.2 days of treatment, the urine volume began to rise and the serum creatinine was gradually decreased (Figure 3). Ten episodes of AR with an antibody response (mixed with cellular response) were completely reversible and one was partially reversible. The function of all grafts was stable with a mean serum creatinine of 1.12 ± 0.32 mg/dl during 13.8 ± 3.5 months (range: 10–21 months) of follow-up (Table 2).

Complications

Complications with severe infection were not observed during the follow-up. One female patient developed post transplant diabetes a month following transplantation, which was accompanied by a urinary infection caused by *Escherichia coli*. The patient recovered after anti-infection therapy with cefoperazone (4 g/day for 7 days) and converting from Tac to CsA. The graft function of this patient was stable during the follow-up.

DISCUSSION

The present study has shown that in Chinese patients receiving a first renal allograft with a negative CDC crossmatch and low PRA, a combination of Tac and MMF, in the absence of IA or PPH, can treat early mixed cellular and humoral rejection successfully with the prevention of graft loss. This finding seems contrary to the current experience with AHR; however, it does support our prediction and is in accordance with our previous observations.[12]

A total of 11 out of 160 (7%) Chinese renal allograft recipients developed AHR during the first 2 weeks post transplantation, all of them mixed with cellular rejection (ranged from Banff borderline to Banff IIB). This incidence rate is comparable to that observed in non-Asian populations.[2,7,11] However, a significantly larger proportion of patients with AHR in the present study were female who had previously been pregnant. This agrees with recent data showing that pregnancy increases the risk of sensitization to transplants in women.[21,22] Thus, we suggest that the combination of Tac-MMF should be used as the optimal initial immunosuppressive protocol in this transplant patient population.

The established treatment for AHR consists of the combined use of IA or PPH in addition to MMF, Tac, and/or IVIG.[6–9] Although these therapies are effective, the use of techniques such as IA and PPH are associated with high costs and development of severe infections.[10–12] However, few studies have examined the role of Tac-MMF alone in the

treatment of AHR. In a recent retrospective study,[12] we reported that the use of Tac-MMF rescue therapy reversed AHR in Chinese renal allograft recipients, with significantly reduced infectious complications compared with IA. These findings are completely consistent with the results from the current prospective study, and the success rates of both studies are similar to those obtained with the additional use of PPH.[7,17]

AHR is a serious complication for transplant patients and can lead to graft loss. Therefore, it may seem surprising that the present study shows that AHR together with cellular rejection can be resolved using pharmacological measures alone, without the need for PPH or IA. Actually, it is not completely unreasonable that Tac-MMF can reverse AHR. Tac-MMF has been effective in the treatment of steroid-resistant AR for many years, and reverses about 60% refractory (even antibody-resistant) rejection cases. We believe some of them might be antibody-mediated AR. In 1998, Pascual[17] reported a cohort of AHR reversed by Tac-MMF combined with PPH. Although PPH was used in their protocol, they noticed that Tac-MMF played a critical role in the reversal of AHR. It is clear that Tac-MMF suppresses the production of new DSA; that is why most attempts to treat AHR by removing DSA with PPH were unsuccessful in the pre-cyclosporine era.[23] A previous study had already proved that MMF can inhibit *in vitro* antibody production by B cells.[24] This evidence suggests that it is Tac-MMF other than IA or PPH which is necessary in the treatment of AHR. Indeed, we are not the first to treat AHR without IA or PPH; Nickeleit *et al.*[13] and Koo *et al.*[14] reported that antilymphocytic preparation without IA or PPH can effectively rescue C4d-positive rejection with pronounced allograft dysfunction. As renal allograft recipients from eastern countries usually need lower doses of immunosuppressants than Caucasian recipients, the potential differences based on ethnicity may also contribute to the good outcome of this cohort. All patients had received bolus steroid; however, it took 16.19 ± 6.16 days for the graft function begun to return. Maybe steroid had some contributory effect; however, we believe that the reversal of rejection was mainly due to MMF and Tac.

Our retrospective studies[12,15] also suggested that AHR developed during the first 2 weeks is usually associated with a good outcome, and it is late AHR that is associated with greater graft loss. These may account why we observed such good outcomes in this cohort. However, our data[15] have proved that this protocol is not as effective in the late AHR episodes. This study excluded patients with detectable serum anti-endothelial cell antibodies, which have been associated with a poor prognosis.[25] Kidney transplants were performed on the basis of a negative pre-transplant CDC crossmatch in our center, and we also preferred to exclude patients with high pre-transplant PRA from the waiting list. Thus, patients may have experienced less intense AHR compared with other studies. Indeed, the mean peak PRA level of patients in the current study ($21.5 \pm 5.3\%$ (s.e.)) is much lower than that reported for other transplant populations ($\sim 50\%$).[7–9,26] This investigation also proved that very low levels of anti-donor antibodies can be associated with the occurrence of severe AHR post transplant.[6,27]

It is perhaps counterintuitive that patients who were already receiving Tac-MMF went on to develop AHR. However, evidence from the present study suggests that the DSA might have existed before the initial treatment. Thus, AHR could have developed in the time required for Tac and MMF to achieve their effective concentrations. Secondly, the time taken for the patients' renal function to recover (16.2 ± 6.2 days) correlates with the half-life of serum IgG, the main component of DSA in patients with AHR.[28] Thus, it appears that the combination of Tac-MMF acts by inhibiting the *de novo* production of DSA. These data also suggest that it might be unwise to excise the graft within 20 days after the onset of rejection, as graft function may still recover.

Certainly, it would be more reasonable to mitigate the damage or levels of the antibodies with interventions such as PPH, IA, or intravenous Ig. Although we do not exclude such strategies, they are very expensive especially for patients in developing countries. Our study provides a new alternative for those who cannot afford such strategies.

We want to emphasize that all episodes of AHR in this cohort were mixed with cellular manifestations (ranging from Banff borderline to Banff IIB) and occurred in patients with an initially negative CDC crossmatch and low levels of PRA. This accords with a former report[29] showing that less than 5% of cases of C4d-positive AHR met the diagnosis of so-called 'pure humoral rejection'. We also noticed that the incidence of tubulitis and interstitial infiltrations were not common in this cohort, even in patients with Banff type II rejection. This may be because most of them had received one to three dose of bolus steroid treatment before the biopsy. It appeared that the clinical course of patients in this group was similar to that of patients with acute tubular necrosis associated with ischemia–reperfusion injury. However, acute tubular necrosis is usually associated with activation of the alternative, but not the classical, pathway of complement.[30] C4d staining was not observed in ischemia or ischemia–reperfusion injury in perioperative renal transplant biopsies.[31] Both the clinical and histological features observed in this group proved that rejection was antibody-mediated.

However, 'pure' AHR, although rare, may respond in a different way to anti-rejection therapy than mixed cellular and humoral C4d-positive rejection. So whether this protocol is suitable for the treatment of the 'pure' AHR remains to be determined. Similarly, this experience does not apply to patients intentionally transplanted across a positive crossmatch, and may not apply to crossmatch negative patients with higher level of pre-transplant PRA.

In conclusion, our study has revealed that the combination of Tac and MMF, in the absence of PPH or IA, might be an effective and inexpensive method to treat early mixed cellular and humoral C4d-positive rejection in renal allograft

recipients, at least for these non-sensitized patients without evidence of anti-endothelial cell antibodies. The effect for sensitized cases and 'pure' AHR remains to be determined. As the incidence of AHR is very low, a large multicenter study would be helpful to prove these findings.

MATERIALS AND METHODS

Patients

One hundred and sixty cadaveric renal allograft recipients transplanted between January 2004 and February 2005 in Jinling Hospital, Nanjing University School of Medicine were enrolled in this study. A previous study[25] had found that AHR with positive serum anti-endothelial cell antibodies was associated with a poor graft outcome. Therefore, patients with detectable serum anti-endothelial cell antibodies were excluded from this study. Informed consent was obtained from all patients, and the Human Subjects Committee of Jinling Hospital, Nanjing University School of Medicine approved all study protocols.

Patients were monitored for AHR episodes according to clinical manifestations, histological features, C4d staining, and anti-DSAs detection. Once there were signs of AR, renal allograft biopsy was performed immediately. We used the followed criteria to include patients: (1) evidence of severe rejection, resistant to steroid therapy; (2) C4d deposition in the PTC area, and (3) typical pathologic features (granulocyte infiltration in glomeruli and PTCs). To verify the diagnosis criteria, DSA was assessed by post transplant PRAs and post transplant cytotoxic crossmatch. Steroid-resistant rejection was defined as serum creatinine levels not returning to within 20% of baseline within 5 days after the last methylprednisolone pulse.[16]

Renal allograft pathology and C4d staining

Diagnostic biopsies were performed after the onset of presumed rejection. Two needle biopsy cores were obtained from each renal allograft for morphologic study, and divided into two parts: one for formalin fixation and one for quick-freezing. Hematoxylin and eosin, periodic acid Schiff, Methenamin-Silver, and Masson staining were routinely used in the formalin-fixed tissue. Fresh frozen tissue was analyzed by immunofluorescence microscopy using a conventional panel of antibodies against IgG, IgM, IgA, C3, C4, and C1q. C4d staining was routinely performed on frozen slides, using an indirect immunofluorescence technique with a primary affinity-purified monoclonal antibody (mouse anti-human; dilution, 1:50; 1.5 h incubation at room temperature; Quidel, San Diego, CA, USA) and an fluorescein isothiocyanate-labeled affinity-purified secondary rabbit anti-mouse IgG antibody (1:20; 40-min incubation at room temperature; DAKO, Denmark). Staining was performed according to standard procedures. All biopsies contained at least 10 glomerular and two arterial sections. A positive C4d staining was defined as bright linear staining along capillary basement membranes, involving over half of sampled capillaries according to 2001 and 2003 Banff Meeting.[18,19] Except in two cases (patient 7 and 11), all patients had received one to three doses of bolus steroid treatment (500 mg/day) before biopsy.

Initial immunosuppression

Two primary immunosuppressive protocols were used: CsA, MMF and steroids or Tac, MMF, and steroids. Induction therapy with either daclizumab or basiliximab could also be used. The choice of protocol and use of induction therapy was based on the pre-transplant PRA and crossmatch as well as the patient's wish. The initial dose of MMF was 1.5 g/day, as a previous study had shown that Chinese patients required lower doses of immunosuppressants than are currently indicated for other populations (2 g/day).[32] Calcineurin inhibitors were added when the serum creatinine level decreased to 50% of pre-transplant (1 h before operation) levels. Tac was initiated at 0.6 mg/kg day^{-1}, CsA was initiated at 4 mg/kg day^{-1}, and both were increased gradually accordingly with the recovery of graft function. The maintenance doses of Tac and CsA were adjusted to trough levels: 6–12 ng/ml during the first 6 months and 4–8 ng/ml during the second 6 months for Tac, and 150–250 ng/ml during the first 6 months and 100–200 ng/ml during the second 6 months for CsA. A standard corticosteroid tapering regimen was used, consisting of an intravenous bolus of methylprednisolone (500 mg) on days 0–2, followed by oral prednisone 80 mg/day on day 3, and then tapered 10 mg/day increments to 20 mg/day. The dose of corticosteroid was then tapered slowly to 5 mg/day thereafter.

PRA and lymphocytotoxic crossmatch

Before transplantation, human lymphocyte antigen-I and human lymphocyte antigen-II antigens were routinely detected with flow cytometry analysis (Flow-PRA) in the method described by Pei *et al.*[33] Sera with greater than 10% flow-PRA class I and/or II reactivity were considered anti-human lymphocyte antigen antibody-positive. Antibody screening was also performed by CDC methods using the National Institutes of Health technique with undiluted complement without wash; a dead cells count less than 10% was considered CDC negative. Kidney transplants were performed on the basis of a negative pre-transplant CDC crossmatch with donor lymphocytes. The above-mentioned two methods were also used to determine the level of DSAs after transplantation. Donor spleen lymphocytes were stored for future detection of post transplant donor-specific crossmatch.

Treatment of AHR

During the first 2 weeks, once a rejection episode had occurred, bolus corticosteroid therapy (methylprednisolone 500 mg optical density for 3 days) was selected as first-line treatment. Concomitantly, all the patients were given MMF (1.5 g/day) and Tac (trough levels maintained at 8–15 ng/ml). For patients being treated with Tac, MMF, and steroids as primary immunosuppression, the dose of Tac was increased so that trough levels were maintained at 8–15 ng/ml. If patients needed dialysis, continuous veno-venous hemofiltration was performed. No other rescue methods such as anti-thymocyte globulin, IA, or PPH were used. We use complete reversal, partial reversal, controlled, and lost graft to demonstrate the outcome of graft, just as we used in a previous study.[15] Complete reversal meant that graft function recovered to the normal range (1.2 mg/dl in our department) within 1 month of rescue therapy; partial reversal meant that graft function improved but did not recover to the normal range within 1 month; controlled meant that graft function did not improve but remained stable without dependence on dialysis in the first month; graft loss meant that the recipient returned to dialysis owing to rejection within 1 month after the rejection.

Statistics

Results are expressed as the mean $\pm$ s.d. The analysis was performed using Stata 6.0 (Stata Corporation, College Station, TX, USA). A χ^2 test was used for testing the significance of categorical variables, and $P < 0.05$ was taken as being significant.

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