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Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status

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Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status.

Background. Both peritubular capillary (PTC) C4d deposition and macrophage/monocyte (MO) infiltration in acute rejection (AR) have separately been shown to be associated with reduced graft survival and recently were demonstrated to be closely correlated in AR. Whether MO infiltration is an independent predictor of graft outcome is uncertain.

Methods. All patients with biopsy-proven AR (over a 3-year period) were included ($N = 96$). All biopsies ($N = 121$) were graded according to the Banff 97 criteria and immunohistochemically stained for C4d and MO (CD68). The primary outcome was glomerular filtration rate (GFR) <30 mL/min 1-year posttransplant as estimated by the Modified Diet in Renal Disease (MDRD) Formula. Secondary outcomes at 2 and 4 years' posttransplant were also explored. A variety of clinical and biopsy variables were statistically analyzed to establish univariate predictors of graft outcome. Stepwise multivariate logistic regression modeling was applied to determine independent predictors of outcomes.

Results. There was a close correlation between PTC C4d and glomerular MO infiltration ($P < 0.001$). Univariate predictors of primary outcome (GFR <30 mL/min 1-year posttransplant) included mean glomerular MO count ≥1.0 MO/glomerulus ($P = 0.014$), female sex ($P = 0.02$), higher peak ($P = 0.005$), and pretransplant ($P = 0.003$) panel-reactive antibody levels, cadaveric donor ($P = 0.006$), transplant glomerulitis ($P = 0.004$), and longer cold ischemic time (CIT) ($P = 0.002$). Mean MO/glomerulus ≥1.0 [OR 10.3 (1.23, 87.1)], female sex [OR 5.27(1.31, 21.1)], and CIT [OR 1.14 (1.06, 1.25)] were identified as independent predictors of adverse graft outcome. Furthermore, mean MO/glomerulus ≥1.0 independently predicted poor renal function at 2 years [OR 3.89 (1.19, 12.70)] and 4 years [OR 4.03 (1.22, 13.28)] posttransplant.

Conclusion. The results demonstrate that glomerular MO infiltration is an independent predictor of worse outcomes posttransplant following acute renal allograft rejection.

Key words: kidney transplant, acute rejection, monocyte, C4d, prognosis.

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Acute renal allograft rejection is associated with decreased graft survival [1–4]. Markers of antibody-mediated (humoral) rejection (AHR) identify patients at high immunologic risk who are more likely to have a worse graft outcome [4, 5]. These markers include peritubular capillary (PTC) staining for complement fragment C4d, neutrophilic (PMN) infiltrate, arterial fibrinoid necrosis, vascular thrombosis, acute tubular injury, infarction, and glomerulitis [6, 7]. In these studies, many of the biopsies from patients with acute humoral rejection (AHR) also demonstrate the histologic features of acute cellular rejection (ACR) as defined by the Banff and Cooperative Clinical Trials in Transplantation criteria (CCTT) [6–8].

Rejection episodes demonstrating the features of AHR have been shown to carry a poor long-term prognosis [6, 9–11] and are often resistant to conventional antirejection therapies [4, 8, 12, 13]. More recently, unconventional measures targeting suppression of recipient humoral immunity, including plasmapheresis (with IVIG administration or tacrolimus-mycophenolate rescue) or immunoadsorption have been demonstrated to reverse some of these rejection episodes, emphasizing the role of AHR in allograft outcomes, even in biopsies also demonstrating features of ACR [12, 14, 15].

Previous studies have demonstrated that glomerular and interstitial monocyte/macrophage (MO) infiltration in acute rejection have a detrimental effect on graft survival [16–26], and are associated with diffuse PTC C4d deposition in acute rejection [27]. In view of the correlation of PTC C4d deposition with reduced graft survival [6, 9–12], it is uncertain whether MO infiltration is a significant independent predictor of poor allograft outcome.

The primary goal of this retrospective study was to test the independent prognostic value of MO infiltration in acute rejection on 1-year renal allograft function. Longer term outcomes at 2 and 4 years' posttransplant were secondarily examined. The results demonstrate that glomerular MO infiltration is an independent predictor of a worse outcome at 1, 2, and 4 years' posttransplant following acute renal allograft rejection.

METHODS

Patients

All renal transplant recipients in British Columbia with biopsy-proven acute renal allograft rejection (Banff Criteria 1997 [28]) between January 1, 1999 and December 31, 2001, in whom the rejection episode was within 12 months of transplant and sufficient tissue was available for histologic and immunohistochemical analyses were included. Of 106 potential subjects, 5 who died within the first year of transplant and 5 with inadequate number of glomeruli (<5) for glomerular MO count were excluded. In total, 121 biopsies from 96 subjects were examined.

Recipient and donor data were compiled primarily from the British Columbia Transplant Society renal transplant database and the Patient Registration and Outcome Management Information System (British Columbia). Chart review was performed when specific information was not available from the databases.

Histologic analyses

Renal biopsies were divided into 3 portions for light microscopy, electron microscopy, and immunohistochemistry. For light microscopy, tissue was fixed in either B5 fixative (122 biopsies) and embedded in paraffin or Karnovsky's fixative (4 biopsies) and embedded in polyglycol methacrylate. Sections embedded in paraffin were cut at 2 μm and stained with hematoxylin-eosin (H&E), periodic acid–Schiff (PAS), and periodic acid-silver methenamine (PASM). Sections embedded in polyglycol methacrylate were cut at 1 μm and stained with H&E and PASM. The biopsies were examined by one of the authors (A.B.M.).

Immunohistochemical analyses

The avidin-biotin peroxidase complex procedure for antibody localization was used. Acetone-fixed sections of snap-frozen renal tissue were stained with commercially available mouse monoclonal antibodies specific for complement split factor C4d (Quidel, San Diego, CA, USA). Snap-frozen sections from biopsies of membranous glomerulonephritis, which show strong glomerular staining for C4d served as positive controls for C4d. B5-fixed paraffin-embedded sections were stained with commercially available mouse monoclonal antibodies specific for CD68 (a marker for MO) (Dako, Carpinteria, CA, USA). For biopsies in which the histologic portion had been embedded in polyglcol methacrylate, acetone-fixed sections of snap-frozen renal tissue were stained for CD68 with commercially available mouse monoclonal antibodies (Dako). Lymph node tissue was used as positive controls for CD68. Negative controls consisted of cases of thin basement membrane disease. Additional control studies were performed by omitting the primary monoclonal antibody in the staining procedure and by using an irrelevant mouse monoclonal antibody as the primary antibody.

The biopsies were evaluated by one of the authors (A.B.M.). A biopsy was considered positive for C4d if PTC showed either diffuse (>50% 0f PTC) or focal (<50% of the PTC) circumferential staining for C4d. In those biopsies with focal reactions, more than 10 PTC had to show circumferential staining for C4d for a biopsy to be scored as positive [29].

Clinical follow-up and monitoring

The primary outcome was glomerular filtration rate (GFR) <30 mL/minute at 12 months' posttransplant, including graft failure**,** as estimated by the Modified Diet in Renal Disease (MDRD) Formula [30], which has validity in transplant recipients [31, 32]. This particular threshold was chosen based on the current K-DOQI classification of Chronic Kidney Disease (CKD) [33] and corresponds to stage 4 CKD, in which progression to end-stage CKD (stage 5) is greater, preparations are made for dialysis or transplant, and the prevalence of renal-related comorbidities is high.

Secondary outcomes were MDRD GFR <30 mL/minute at 24 and 48 months' posttransplant. By 24 months, 2 subjects had been lost to follow-up, leaving 94 subjects for analysis. Eighty-eight subjects had 48 complete months of posttransplant follow-up.

The following clinical parameters were assessed: (*1*) patient and donor ages at the time of renal transplantation; (*2*) recipient gender; (*3*) graft type (living related, living unrelated, cadaveric); (*4*) cold and warm ischemic times; (*5*) serum panel-reactive antibody (PRA) levels, both historic and at the time of transplantation; (*6*) number of previous transplants; (*7*) number of HLA mismatches (0 to 6) between donor and recipient (A, B, and DR); (*8*) presence or absence of delayed graft function defined as a failure of serum creatinine (Cr) to fall below 50% of initial Cr by 1 week posttransplant; (*9*) time to rejection; and (*10*) baseline and follow-up serum Cr levels and patient weights sufficient for calculating MDRD GFR at all time points of interest. Neither HLA antibody monitoring nor flow cytometric crossmatching concurrent to AR was routinely performed at our institution during the era of this study.

Initial immunosuppression was achieved using a standard triple therapy regimen of methylprednisolone, 1 mg/kg/day for 3 to 5 days, then prednisone (0.7–1 mg/kg/day tapering to 0.3 mg/kg/day by 2 weeks' posttransplant and 0.15 mg/kg/day by 6 months) (all patients), cyclosporine (CSA), 9.0 mg/kg/day adjusted for trough levels 425 to 500 μg/mL for the first 30 days, then tapered gradually to levels of 100 to 150 μg/L for long-term maintenance (70 patients), or tacrolimus (TAC), 0.12 to 0.15

mg/kg/day adjusted for trough levels 10 to 15 μg/mL for the first 30 days then tapered gradually to levels of 5 to 8 μg/mL for long-term maintenance (22 patients), and mycophenolate mofetil (MMF), 2000 mg/day (97 patients) or azathioprine (AZA) 1 mg/kg/day (2 patients). Induction therapy was used in 11 patients at high immunologic risk (PRA >30%, second or greater transplant) and consisted of a 7-day course of therapy with antilymphocyte antibodies (OKT3; Ortho Biotech, Raritan NJ, USA, or ATG; Genzyme, Cambridge, MA, USA), 5 mg/day. All rejection episodes were treated with methylprednisolone for 3 to 6 days (total dose range 1500 mg to 4000 mg). Patients with Banff 97 grade 2A or greater or steroid resistant rejections were treated with OKT3 5 mg/day or ATG for 7 to 10 days. One C4d+ patient had plasma exchange for 2 rejection episodes, with a total of 10 exchanges for each episode, and IVIG following the plasma exchange for the second episode. Another C4d+ patient with features suggestive or AHR received IVIG alone.

Quantitative analysis

All biopsies were scored according to the Banff 97 criteria to determine the type and grade of the rejection reaction. MO and T cells were counted in all glomeruli in each biopsy and expressed as the number of cells per glomerulus. A threshold of greater than or equal to 1 MO per glomerulus was used to discriminate between high and low glomerular monocyte counts. The number of cortical interstitial (CI) MO per high power field (hpf) (×40 objective with an object area diameter of 0.5 mm and an area of 0.196 mm^2) in each biopsy was determined by counting the number of CI MO and cortical hpfs using a high power objective (×40) and dividing the number of cells by the number of cortical hpfs. For the quantitative analyses at least 5 glomeruli were available for evaluation.

Statistical analysis

Descriptive statistical values are presented as mean ± SD or as median: range depending on normality of the underlying distribution. Continuous variables were compared using the *t* test or the Wilcoxon rank sum test where appropriate. Categorical variables were compared using the χ^2 test or Fisher exact test where appropriate. A *P* value of less than 0.05 for two-sided univariate tests was considered significant. Stepwise multivariate logistic regression modeling was applied to the outcomes of MDRD GFR ≤30 mL/minute at 12, 24, and 48 months' posttransplant with *P* values for exclusion and inclusion in the models 0.15 and 0.10, respectively. All potential biologic confounders were examined in the model (cadaver donor, recipient sex, peak PRA, cold ischemic time, total number of HLA mismatch, delayed graft function, Banff grade, and C4d staining) and those variables that were significant univariate predictors of each outcome were forced into the model. Multiple threshold and categorical models for defining high glomerular MO counts were explored; the threshold of glomerular MO count of ≥1 was used as it provided the greatest discriminatory capability in the primary outcome multivariable model with area under the ROC curve of 89%. Notably, results were similar irrespective of the threshold used. Potential effect modification between glomerular MO count and all model variables was individually tested for and none was found. The most parsimonious model with the greatest discriminatory value as determined by ROC analysis is presented.

RESULTS

Renal biopsy findings

The 96 patients had 121 biopsies demonstrating various grades of acute rejection. Eighty-five had 1 biopsy, 16 had 2 biopsies, and 1 patient had 4 biopsies. For those with more than 1 biopsy the highest Banff 97 grade and the highest glomerular and CI MO scores were used for the statistical analysis. All but 15 biopsies had 10 or more glomeruli. Twelve biopsies had 9 glomeruli, 2 had 8 glomeruli, and 1 had 7 glomeruli. All but 6 biopsies had at least 2 large arteries for evaluation. One artery was present in 3 of the biopsies, while 3 other biopsies did not contain a large artery. In 31 patients the renal biopsy was suspicious for acute rejection. For 37 and 27 patients the highest Banff 97 grades were 1 (interstitial rejection only) and 2 (vascular rejection), respectively. One patient showed severe acute vascular rejection (Banff 97 grade 3). For analytical purposes this patient was grouped with those showing less severe vascular rejection (Banff 97 grade 2). Transplant glomerulitis, expressed in this study as Banff 97 Classification g score ≥1.0 [28], was noted in 31 biopsies. None of the biopsies examined showed chronic allograft nephropathy.

PTC C4d staining was observed in at least 1 biopsy from 27 patients (Table 1). This was diffuse in 16 and focal in 11. All biopsies exhibited varying degrees of glomerular, arterial, and arteriolar reaction for C4d.

Glomerular MO infiltration (Fig. 1) was observed in at least 1 of the biopsies from 67 patients. Forty-four of these biopsies had an MO/glomerulus (MO/Glom) score of <1.0 (mean 0.43 ± 0.27 MO/Glom; range 0.1–0.9 MO/Glom), while 23 showed 1 or more MO/Glom (mean 3.5 ± 2.0 MO/Glom; range 1.1–8.4 MO/Glom). Varying numbers of CI MO were present in all of the biopsies (mean 11.9 ± 11.0 MO/hpf; range 0.5–66.6 MO/hpf).

Patient and clinical characteristics

The detailed general characteristics of the entire cohort are presented in Table 1. The male:female ratio was 58:38.

Table 1. Patient population characteristics

Variable [a]	All patients ($N = 96$)	Patients with <1 MO/glomerulus ($N = 73$)	≥1 MO/glomerulus ($N = 23$)	P value
Demographics				
Sex (*N* female)%	38 (40.0)	24 (32.9)	14 (60.9)	0.017
Mean recipient age *years*	45.2 ± 12.4	44.6 ± 12.2	47.1 ± 12.9	0.38
Mean donor age *years*	40.0 ± 15.0	39.3 ± 13.5	42.5 ± 19.0	0.44
Previous kidney transplant *N* (%)				0.13
0	79 (82.3)	63 (86.3)	16 (69.6)	
1	15 (15.6)	9 (12.3)	6 (26.1)	
≥2	2 (2.1)	1 (1.4)	1 (4.3)	
Peak PRA *N* (%)				0.004
0%	77 (80.2)	64 (87.7)	13 (56.5)	
1–29%	11 (11.5)	6 (8.2)	5 (21.7)	
≥30%	8 (8.3)	3 (4.1)	5 (21.7)	
Pretransplant PRA *N* (%)				0.03
0%	82 (85.4)	66 (90.4)	16 (69.6)	
1–29%	9 (9.4)	5 (6.9)	4 (17.4)	
≥30%	5 (5.2)	2 (2.7)	3 (13.0)	
Transplant factors				
Donor type *N* (%)				0.16
Cadaveric	40 (41.6)	27 (37.0)	13 (56.5)	
LUD	25 (26.0)	19 (26.0)	6 (26.1)	
LRD	31 (32.3)	27 (37.0)	4 (17.4)	
Mean number of donor-recipient HLA matches	3.60 ± 1.28	3.51 ± 1.27	3.91 ± 1.31	0.19
Cold ischemic time *hours*	3.58: 1.45–28.0	3.43: 1.45–27.9	7.72: 2.5–24.0	0.05
Delayed graft function *N* (%)	29 (30.2)	18 (24.7)	11 (47.8)	0.07
Immunosuppression *N* (%)				
CsA	32 (33.3)	24 (32.9)	8 (34.8)	1.0
Tacrolimus	64 (66.7)	49 (67.1)	15 (65.2)	
MMF	89 (92.7)	67 (91.8)	22 (95.7)	1.0
Azathioprine	5 (5.21)	4 (5.5)	1 (4.4)	
OKT3/ATG therapy *N*	16 (16.7)	12 (16.4)	4 (17.4)	0.76
OKT3/ATG induction *N*	4 (3.1)	1 (1.37)	2 (8.7)	0.14
CNI[b] level at time of rejection *N* (%)				0.73
Low	49 (51.0)	38 (52.1)	11 (48.8)	
Appropriate	31 (32.3)	22 (30.1)	9 (39.1)	
High	16 (16.7)	13 (17.8)	3 (13.0)	
Rejection				
Time to acute rejection *days*	13: 3–364	14: 3–364	11: 5–187	0.84
Cr at time of rejection μ*mol/L*	239: 117–856	234: 117–856	256: 155–644	0.36
Increase in Cr from baseline μ*mol/L* ($N = 74$ achieved baseline Cr prior to rejection)	49: 10–509	48:10–509	50: 10–274	0.53
% change in Cr from baseline	35.6: 6.4–213	36.8: 6.4–213	36.6: 6.8–170	0.74
Banff class *N* (%)				0.38
Suspicious	31 (32.3)	22 (30.1)	9 (39.1)	
I	37 (38.5)	31 (42.5)	6 (26.1)	
II	28 (29.2)	20 (27.4)	8 (34.8)	
C4d staining *N* (%)				<0.001
Negative	69 (71.9)	65 (89.0)	4 (17.4)	
Focal	11 (11.5)	7 (9.6)	4 (17.4)	
Diffuse	16 (16.7)	1 (1.4)	15 (65.2)	
Transplant glomerulitis[c] *N* (%)				<0.001
g score 0	65 (67.7)	60 (82.2)	5 (21.7)	
1	17 (17.7)	7 (9.6)	10 (43.5)	
2	7 (7.3)	2 (2.7)	5 (21.7)	
3	7 (7.3)	4 (5.5)	3 (13.0)	
CI MO/hpf [d]	8.4: 0.5–66.6	7.3:0.5–66.6	12.3:0.7–36.0	0.11
Clinical outcomes				
MDRD GFR <30 mL/min 12 months' posttransplant *N* (%)	17 (17.7)	9 (12.3)	8 (34.8)	0.025
MDRD GFR <30 mL/min 24 months' posttransplant *N* (%) ($N = 94$ available)	19 (16.2)	9 (12.5)	10 (45.5)	0.001
MDRD GFR <30 mL/min 48 months' posttransplant *N* (%) ($N = 88$ available)	23 (26.1)	13 (18.6)	10 (55.5)	0.005
% decrease in Cr 2 weeks' postrejection	15.8: 74.6–1026	19.2: 74.6–793	6.7: 76.3–1026	0.07
Graft failure at 12 months *N* (%)	8 (7.9)	3 (4.1)	5 (21.7)	0.018

[a]Continuous variables expressed as mean ± SD or median:range as indicated.
[b]CNI is calcineurin inhibitor.
[c]Transplant glomerulitis expressed as g score ≥1.0 (Banff 97 classification).
[d]CI MO/hpf is cortical interstitial monocytes per high-power field.

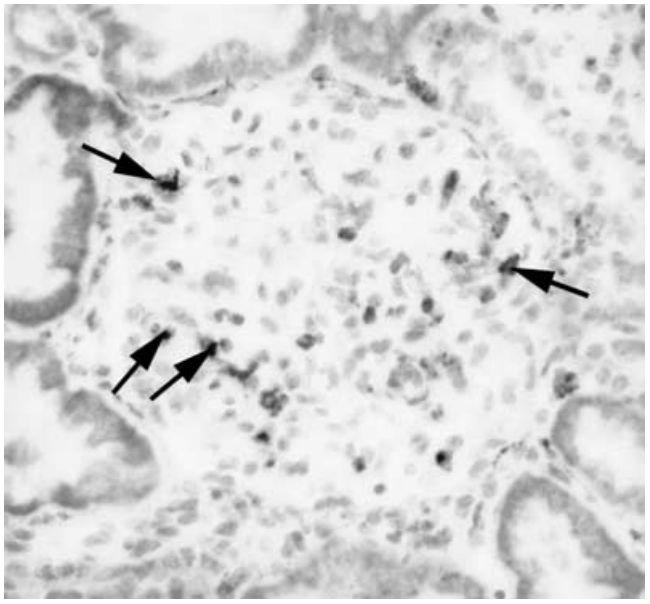

Fig. 1. Immunohistochemical demonstration of CD68+ cells. The glomerulus contains a number of CD68+ cells, some of which are indicated by arrows (×250).

The mean recipient age was 45.2 ± 12.4 years (range 22–79 years). The age range of the donors was 3 to 67 years, with a mean of 40.0 ± 15.0 years. Seventeen patients had a previous transplant. A peak PRA ≥30% (highest on record) was noted in 8 patients. Five patients had an immediate pretransplant PRA ≥30%. Forty patients received a cadaveric allograft, while 56 obtained a living donor graft (25 living unrelated, 31 living related). The mean number of HLA donor-recipient mismatches was 3.60 ± 1.28. Cold ischemic time (CIT) varied from 1.45 hours to 28.0 hours with a median of 3.58 hours. Delayed graft function occurred in 29 patients. CSA was given to 32 patients, while 64 received TAC. MMF was used in 89 cases, while 5 patients received AZA. Sixteen patients were treated with antilymphocyte antibodies for rejection. Calcineurin inhibitor levels at the time of rejection were low in 49 patients, high in 19, and within the therapeutic range in 31. The time to rejection ranged from 3 to 364 days posttransplantation with a median of 13 days. The median magnitude of the rise in Cr at the time of rejection from baseline values was 49 μmol/L (range 10.0–509 μmol/L).

Seventeen patients had a MDRD GFR <30 mL/min at 1 year posttransplant, including 8 experiencing graft failure. The median time to graft failure was 105 days with a range of 21 to 311 days. An additional 2 patients had an MDRD <30 mL/min by 24 months and 3 more patients developed an MDRD <30 mL/min by 48 months.

Comparison of high and low glomerular monocyte groups

The patients were divided into 2 groups according to level of glomerular monocyte infiltration—those with mean number of MO/glomerulus ≥1.0 (high MO group) and those with <1.0 MO/glomerulus (low MO group). The general characteristics and comparisons between the 2 groups are also presented in Table 1. There were proportionately more women in the high MO group (61% vs. 32.9%, $P = 0.017$). Twenty-one percent of subjects in the high MO group had peak PRA ≥30% compared with only 4.1% in the low MO group and, similarly, 13% of the high MO group had high PRA immediately prior to transplant compared with only 2.7% in the low MO group ($P = 0.004$ and 0.03, respectively). Longer CIT and, related to this, a higher incidence of delayed graft function were seen in the high MO group ($P = 0.05$ and 0.07, respectively) as was a poorer response to therapy as defined by percent change in Cr 2 weeks' postrejection, although this did not reach statistical significance ($P = 0.07$). Number of previous transplants, cadaveric versus living donor, number of HLA mismatches between donor and recipient, and type and level of immunosuppression was not significantly different between the 2 groups. In addition, median time to acute rejection was similar in both groups, as was the mean serum creatinine at the time of rejection and distribution of Banff 97 grades.

Glomerular monocytes and pathology findings

Banff 97 classification did not correlate with glomerular MO count. Transplant glomerulitis expressed as Banff 97 g score ≥1.0 was significantly more prevalent in the high MO group (78.2%) than in the low MO group (17.8%). Higher median CI MO counts were seen in the high MO group (12.3 vs. 7.3), but this difference did not reach statistical significance ($P = 0.11$).

Glomerular monocytes and C4d staining

The presence of glomerular MO is strongly correlated with positive staining for C4d. Eighty-three percent (83%)of high MO biopsies also were C4d positive, while 89% of low MO biopsies were C4d negative ($P < 0.001$) (Table 2). The strong correlation was maintained in those subjects with the primary outcome of interest ($P = 0.027$) (Table 2), as well as those with better graft function at 1 year ($P < 0.001$) (data not shown). Furthermore, the association between these 2 pathologic markers is equally strong in both Suspicious/class I rejections and class II rejections ($P < 0.001$ and $= 0.006$, respectively) (Table 2).

Outcome

Poor allograft function at 1 year [GFR (MDRD) <30 mL/min] was predicted by the glomerular monocyte count. A percentage of subjects (34.8%) with ≥1.0 MO/glomerulus achieved this primary outcome, compared with 12.3% of subjects in the low MO group ($P =$

Table 2. Association of glomerular monocytes with C4d staining

All subjects ($N = 96$)	C4d positive	C4d negative	
≥1 MO/glomerulus	19	4	
<1 MO/glomerulus	8	65	$P < 0.001$
Subjects with MDRD GFR <30 mL/min 12 months' posttransplant ($N = 17$)			
≥1 MO/glomerulus	5	3	
<1 MO/glomerulus	1	8	$P = 0.027$
Subjects with Banff suspicious or class I rejection ($N = 68$)			
≥1 MO/glomerulus	13	2	
<1 MO/glomerulus	4	49	$P < 0.001$
Subjects with Banff class II rejection ($N = 28$)			
≥1 MO/glomerulus	6	2	
<1 MO/glomerulus	4	16	$P = 0.006$

0.025) (Table 1). Additional univariate predictors of the primary outcome are presented in Table 3. Higher peak PRA ($P = 0.005$), higher pretransplant PRA ($P = 0.003$), cadaveric donor ($P = 0.006$), longer CIT ($P = 0.002$), female gender ($P = 0.02$), and g score ($P = 0.004$) were all associated with adverse outcomes. Stepwise forward logistic regression modeling was applied to the primary outcome, adjusting for all significant univariate predictors, as well as potentially important biologic confounders. Higher glomerular MO count [OR 10.3 (1.23, 87.1)], female sex [OR 5.27 (1.31, 21.1)], and longer cold ischemic time [OR 1.14 (1.06, 1.25)] independently predicted adverse 1-year allograft outcome (Table 4).

Transplant glomerulitis was a significant predictor of primary outcome at 1 year (Table 3). Although there was no association between C4d staining and 1-year outcome (Table 3), 47.8% of those with poor outcome at 24 and 48 months had positive PTC staining for C4d compared to 19.7% of other subjects ($P = 0.011$, data not shown). In addition, all significant univariate predictors of the primary outcome were predictive of 24- and 48-month outcomes. Stepwise forward logistic regression modeling was applied to these secondary outcomes, and high glomerular MO count remained independently predictive of 24-month [OR 3.89 (1.19, 12.70)] and 48-month [OR 4.03 (1.22, 13.28)] outcomes. Peak PRA was also independently predictive of outcome at 24 months [OR 2.84 (1.27, 6.34)] and 48 months [OR 3.40 (1.37, 8.42)] (Table 4).

In both C4d positive and C4d negative subjects, subjects with higher glomerular monocyte counts have a greater prevalence of poor 1-year allograft function ($P = 0.008$) (Table 5). Among C4d positive subjects, 26.3% of subjects with high glomerular MO counts achieved the primary outcome, compared with 12.5% of those with low glomerular MO counts. With negative C4d staining, 75% of high MO subjects achieved the primary outcome versus 12.3% of those with low MO biopsies.

DISCUSSION

The results of this retrospective study establish the association between the presence of glomerular MO in acute rejection biopsies and reduced renal allograft survival 1, 2, and 4 years' posttransplant independent of other known predictors of adverse outcome, including histologic type (Banff 97 grade) and PTC C4d status. The occurrence of graft failure at all time points, and slower recovery from acute rejection, was also greater in the high MO group. The results of the present study are consistent with previous demonstrations of the adverse effect by glomerular MO on graft outcome [17, 18, 26].

The present investigation failed to find a significant association between level of glomerular monocyte infiltration and Banff 97 grade. One previous study noted that the highest numbers of glomerular MO were found in cases of vascular rejection [26]. In addition, the results of another study suggested, albeit indirectly, a relationship between transplant glomerulitis, previously shown to be due to glomerular accumulation of MO and T lymphocytes [27, 34, 35] and vascular rejection [29]. However, Harry et al did not note any correlation between glomerular MO infiltration and any particular biopsy change [17]. Differences in patient population and treatment may account for the discrepancy in the results.

The results of the present investigation confirm and extend the previously reported association between glomerular MO infiltration and PTC C4d deposition [27]. In view of this association and the results of previous studies [6, 9–11], especially the one from our institution [9], the failure to demonstrate an independent detrimental effect of PTC C4d on graft outcome was unexpected. The previous investigation from our laboratory involved biopsies of acute rejection done between July 1, 1995 and December 31, 1997 [9], whereas the present retrospective study looked at patients with biopsies done between January 1, 1999 and December 31, 2001, during which time all renal allograft biopsies were immunostained for C4d, there was an increasing awareness of the significance of PTC C4d staining, and there was increased use of both TAC and MMF. Additionally, glomerular MO was not assessed in the previous study [9]. This may account in part for the differences in results with respect to the predictive value of PTC C4d deposition. PTC C4d deposition was univariately predictive of longer-term outcomes; whether a significant independent deleterious effect of PTC C4d deposition on graft outcome over even longer time periods will be demonstrated is uncertain. Finally, in this regard, the results of the present investigation are consistent with those of another study, which did not show

Table 3. Univariate predictors of primary outcome

Variable[a]	Group with MDRD GFR ≥30 at 12 months' posttransplant ($N = 79$)	Group with MDRD GFR <30 at 12 months' posttransplant ($N = 17$)	*P* value
Demographics			
Sex *N* female (%)	27 (31.2)	11 (64.7)	0.02
Mean recipient age *years*	44.5 ± 11.7	48.1 ± 15.3	0.18
Previous kidney transplant *N* (%)			0.16
0	67 (84.8)	12 (70.6)	
1	11 (13.9)	4 (23.5)	
≥2	1 (1.3)	1 (5.9)	
Peak PRA *N* (%)			0.005
0%	67 (84.8)	10 (58.8)	
1–29%	9 (11.4)	2 (11.8)	
≥30%	3 (3.8)	5 (29.4)	
Pretransplant PRA *N* (%)			0.003
0%	71 (89.9)	11 (64.7)	
1–29%	7 (8.9)	2 (11.8)	
≥30%	1 (1.2)	4 (23.5)	
Transplant factors			
Donor type *N* (%)			0.006
Cadaveric	27 (34.2)	13 (76.5)	
LUD	24 (30.4)	1 (5.9)	
LRD	28 (35.4)	3 (17.6)	
Mean number of donor-recipient HLA matches	3.58 ± 1.22	3.70 ± 1.57	0.72
Cold ischemic time *hours*	3.3: 1.45–28.0	11.9: 3.3–25.2	0.002
Delayed graft function *N* (%)	22 (27.8)	7 (41.2)	0.278
Immunosuppression *N* (%)			
CsA	26 (32.9)	6 (35.3)	1.0
Tacrolimus	53 (67.1)	11 (64.7)	
MMF	73 (92.4)	16 (94.1)	0.23
Azathioprine	5 (6.3)	0 (0.0)	
OKT3/ATG therapy	14 (17.7)	2 (11.8)	0.46
OKT3/ATG induction	2 (2.5)	2 (11.8)	0.13
CNI[b] level at time of rejection *N* (%)			0.77
Low	39 (49.4)	10 (58.8)	
Appropriate	27 (34.2)	4 (23.5)	
High	13 (16.5)	3 (17.6)	
Rejection			
Time to acute rejection *days*	11: 3–364	21: 7–356	0.17
% change in Cr from baseline	30.0: 6.4–213	46.2: 11.0–108	0.07
Mean Cr at time of rejection *μmol/L*	273 ± 149	333 ± 158	0.15
Banff class *N* (%)			0.68
Suspicious	25 (31.7)	6 (35.3)	
I	32 (40.5)	5 (29.4)	
II	22 (27.8)	6 (35.3)	
C4d staining *N* (%)			0.69
Negative	58 (73.4)	11 (64.7)	
Focal	9 (11.4)	2 (11.8)	
Diffuse	12 (15.2)	4 (23.5)	
≥1 MO/glomerulus *N* (%)	15 (19.0)	8 (47.1)	0.014
Transplant glomerulitis[c] *N* (%)			0.004
g score 0	59 (74.7)	6 (35.3)	
1	12 (15.2)	5 (29.4)	
2	5 (6.3)	2 (11.8)	
3	3 (3.8)	4 (23.5)	
[4]CI MO/hpf	8.1: 0.5–37.5	11.1: 0.7–66.6	0.38

[a]Continuous variables expressed as mean ± SD or median:range as indicated.
[b]CNI is calcineurin inhibitor.
[c]Transplant glomerulitis expressed as g score ≥1.0 (Banff 97 classification).
[d]CI/MOhpf is cortical interstitial monocytes per high-power field.

a significant difference between C4d positive and C4d negative patients with acute rejection [29].

As transplant glomerulitis is associated with high glomerular MO, it is tempting to use the former as a surrogate for the latter. However, some caution is required as transplant glomerulitis is often the result of glomerular infiltration by both MO and T cells [27] and, in a number of cases, especially in those biopsies that are PTC C4d negative, the predominant infiltrating cell is the T cell (with few MO) [36].

Unlike glomerular MO number, the level of CI MO did not correlate with graft outcome at 1 year (data not

Table 4. Results of stepwise multivariate logistic regression model

Variable	OR	95% CI	*P* value
Primary outcome of MDRD GFR ≤30 mL/min at 1 year posttransplant			
≥1 MO/glomerulus	10.3	[1.23, 87.1]	0.032
Female sex	5.27	[1.31, 21.1]	0.019
Cold ischemic time *hours*	1.14	[1.06, 1.25]	0.001
MDRD GFR ≤30 mL/min at 2 years' posttransplant ($N = 94$)			
≥1 MO/glomerulus	3.89	[1.19, 12.70]	0.025
Peak PRA	2.84	[1.27, 6.34]	0.011
MDRD GFR ≤30 mL/min at 4 years' posttransplant ($N = 88$)			
≥1 MO/glomerulus	4.03	[1.22, 13.28]	0.022
Peak PRA	3.40	[1.37, 8.42]	0.008

Table 5. Percent of subjects with the primary outcome in groups defined by glomerular MO count and C4d staining

	C4d positive	C4d negative	
≥1 MO/glomerulus	26.3% (5/19)	75.0% (3/4)	
<1 MO/glomerulus	12.5% (1/8)	12.3% (8/65)	$P = 0.008$

shown). Several previous studies have shown an association between extent of CI MO infiltration and poor graft survival [16, 19–21, 37]. Differences in patient population, immunosuppression, and treatment of acute rejection may account for this discrepancy.

CONCLUSION

Predictors of the primary outcome by univariate analysis included glomerular MO infiltration (≥1 MO/glomerulus), female sex, peak and pretransplant PRA, cadaveric donor, transplant glomerulitis, and longer cold ischemic times. When adjusted for univariate predictors of the primary outcome, as well as other clinical variables considered to be prognostically significant (including delayed graft function [38, 39], number of HLA mismatches [40, 41], calcineurin inhibitor level, and C4d staining [9]), glomerular MO count ≥1 MO per glomerulus, longer CIT, and female sex remained independent predictors of adverse 1-year allograft function after acute rejection. In addition, glomerular MO count ≥1 MO per glomerulus in AR was independently predictive of poor allograft function as far as 48 months' posttransplant.

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