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Acute renal allograft rejection with intimal arteritis: Histologic predictors of response to therapy and graft survival

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Acute renal allograft rejection with intimal arteritis: Histologic predictors of response to therapy and graft survival.

Background. Acute renal allograft rejection with intimal arteritis is designated by the widely used Banff 97 classification as type 2A or 2B depending on the extent of arteritis, without regard to interstitial inflammation or tubulitis. We examined whether the distinction between type 2A and 2B is relevant to short- and long-term clinical outcomes, and if outcomes in this subset of acute rejection also are affected by tubulitis, interstitial inflammation, and several additional histologic and clinical parameters.

Methods. Pathology records were searched to identify cases of acute renal allograft rejection with intimal arteritis diagnosed between January 1985 and September 2000. For each case, the patient's chart was reviewed to determine the response of the rejection episode to therapy, type(s) of therapy given, and length of graft survival. All biopsies were reviewed and Banff acute and chronic indices recorded by a pathologist blinded to these data. Biopsies not showing type 2A or 2B rejection were excluded, as were repeat biopsies from the same patient and cases with recurrent glomerular disease, viral infection, donor-specific antibodies, or more than mild chronic change.

Results. The initial response to anti-rejection therapy was significantly worse in patients with type 2B acute rejection ($N = 29$) than in those with type 2A ($N = 102$) by univariate and multivariate analyses, despite more aggressive treatment of type 2B rejection. In a Cox proportional hazards model the hazard ratio for graft failure for 2B versus 2A was 1.9 ($P = 0.05$), but this was not significant when adjusted for the initial response to therapy. Cases with minimal or mild tubulitis responded better to therapy than those with moderate or severe tubulitis, although graft survival was not significantly affected by the tubulitis score.

Conclusions. The distinction between types 2A and 2B acute rejection in the Banff 97 classification has significant prognostic value with regard to both short- and long-term clinical outcomes, although the difference in long-term graft survival is mainly related to the initial response to therapy. Reports of biopsies showing type 2A or 2B rejection also should specify the degree of tubulitis present, as the latter may significantly influence the initial response to therapy.

Key words: kidney transplant, Banff 97, tubulitis, acute vascular rejection, acute cellular rejection.

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Acute rejection is manifest on renal transplant biopsy as mononuclear inflammatory cell infiltrates in one or more sites: in the cortical interstitium and tubules (tubulitis), beneath the endothelium of arteries (intimal arteritis or endothelialitis), and infrequently within both the arterial intima and media (transmural arteritis) with or without associated fibrinoid necrosis of the vessel wall. Evidence from a number of studies indicates that these different histologic lesions of acute rejection correlate with the reversibility of a rejection episode by treatment with corticosteroids and/or other anti-rejection drugs, and with long-term survival of the allograft. Overall, lesions characterized by interstitial inflammation and tubulitis alone have the greatest likelihood of reversibility and best prognosis for long-term graft survival, those with transmural arteritis and/or arterial fibrinoid necrosis the poorest degree of reversibility and graft survival, and lesions with intimal arteritis an intermediate prognosis with regard to both clinical parameters [1–8].

These clinico-pathologic correlations have led to the development of a number of grading schemata for acute rejection on renal transplant biopsy, the most widely used of which is the Banff 97 working classification [9]. Banff 97 is a modification of the original (1993) Banff schema [10] that also incorporates elements of a second grading system (the NIH/CCTT system [2]), including the separation into distinct types (or grades) of acute rejection of lesions with interstitial inflammation and tubulitis but without vascular involvement (type 1), with intimal but not transmural arteritis (type 2), and with transmural arteritis/fibrinoid necrosis (type 3).

In the Banff 97 classification, biopsies showing intimal (but not transmural) arteritis are designated as showing type 2B acute rejection if there is severe intimal arteritis

(Banff index v2) comprising >25% of the luminal area of at least one artery, and as type 2A acute rejection if the degree of intimal arteritis is less than this (Banff index v1). This distinction between type 2A and 2B rejection is somewhat arbitrary, although in a recent study of 36 patients with type 2A and 18 patients with type 2B rejection graft loss was significantly greater in the 2B group [5]. The designation of a biopsy as showing type 2A or type 2B acute rejection by the Banff 97 schema is also completely independent of the degree of interstitial inflammation and tubulitis present, although there is some evidence suggesting that the latter parameters, particularly tubulitis, may influence the outcome of acute rejection episodes with intimal arteritis. In a study in the pre-cyclosporine era, Matas et al found that seven of eighteen grafts with moderate acute vascular rejection (intimal arteritis) and moderate-to-severe interstitial infiltrate were lost within the first year, although a sufficiently large number of grafts showing intimal arteritis with little or no interstitial inflammation were not available for comparison [4]. Schroeder et al found that among 29 patients with acute vascular rejection characterized by intimal arteritis treated with OKT3, the poorest clinical outcome was in the group of 9 patients with moderate-to-severe interstitial inflammation and marked tubulitis in addition to the vascular lesion [11]. Furthermore, Nickeleit et al found that in their subset of acute rejection episodes with intimal arteritis, increasing tubulitis (determined as the number of involved tubules in the most affected 10 consecutive high-power microscopic fields), but not interstitial inflammation, appeared to correlate with a greater graft failure rate within the first year post-transplantation [6].

This study retrospectively examined 131 cases of acute rejection with intimal arteritis in order to specifically address if the distinction between types 2A and 2B rejection is relevant to short-term (reversibility of the rejection episode with anti-rejection therapy) and long-term (graft survival) clinical outcomes. We also examined if within this subset of acute rejection these outcomes are affected by the degree of interstitial inflammation, tubulitis, and a number of additional pathologic and clinical parameters.

METHODS

Patient selection and data collection

Computerized records of the Department of Pathology, Johns Hopkins Medical Center were searched to identify all renal transplant biopsies potentially showing intimal arteritis that were interpreted from January 1985 through September 2000. The individual biopsy reports were then reviewed to eliminate all repeat biopsies (of the same allograft or of a subsequent graft in the same patient), as well as cases without intimal arteritis or with transmural arteritis, and those showing recurrent glomerular disease, viral (polyomavirus, cytomegalovirus) infection, or post-transplant lymphoproliferative disease. Twelve patients (9 with type 2A and 3 with type 2B acute rejection by the Banff 97 classification) had documented donor-specific antibodies, and also were eliminated from the study. For the remaining 185 biopsies, clinical records were then reviewed and the following information was recorded: patient age and gender, date of transplant and post-transplant day on which the biopsy was done, type of transplant (living-related, living-unrelated, or cadaveric), baseline serum creatinine prior to the rejection episode prompting the biopsy or if the graft had inadequate function between the time of transplantation and the biopsy (delayed graft function; DGF), baseline immunosuppressive regimen, type(s) of anti-rejection therapy given, post-biopsy course including the lowest stable (for 2 or more daily determinations) serum creatinine within 20 days of the initiation of therapy and if a subsequent biopsy was done, most recent follow-up and serum creatinine at that time, and date of graft failure (re-initiation of dialysis) if appropriate.

Pathologic review of biopsies

Following the review of all clinical records, each biopsy was assigned a number and all clinical data were recorded in a table in which only these numbers were present as identifiers. Microscopic slides from each biopsy were removed from the files and labeled with these numbers prior to review.

The slides from each biopsy [routinely stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS), methenamine silver, and Masson's trichrome] were reviewed by a renal pathologist (M.H.) who was blinded from the clinical data. All but two biopsies were needle biopsies. For each biopsy, the number of tissue cores (not including any that were all medulla; the two wedge biopsies were each counted as a single core), the number of arteries present and the number showing intimal arteritis (v1 or v2), as well as the following parameters were recorded: type of acute rejection by the Banff 97 classification, and semiquantitative indices of acute glomerulitis (g), interstitial inflammation (i), tubulitis (t), arteritis (v), chronic allograft glomerulopathy (cg), interstitial fibrosis (ci), tubular atrophy (ct), fibrous intimal thickening of arteries (cv), arteriolar hyaline thickening (ah) and mesangial matrix increase (mm). Each index was graded from 0 to 3 (absent or minimal to severe) according to specific guidelines established in the Banff 97 schema [9]. As some biopsies with minimal or mild interstitial inflammation did show more extensive interstitial edema, an "edema score" also was determined for each biopsy, based on an estimate of the fraction of cortical tissue with edema: 0, <10%; 1, 10–25%; 2, 26–50%; 3, >50%. Findings of acute tubular injury not associated with apparent foci of acute

rejection and histologic evidence of cyclosporine/tacrolimus nephrotoxicity [12, 13] were recorded when present. On the basis of this review 19 biopsies were excluded from the study: 10 did not show lesions believed to be diagnostic of intimal arteritis, 2 showed transmural arteritis, and 7 did not have adequate tissue for evaluation of the above indices. As this study was designed to consider only the outcome of acute rejection episodes, we also eliminated from the study an additional 26 biopsies showing more than mild chronic change, as defined by a chronic allograft nephropathy (CAN) score (cg + ci + ct + cv + ah + mm) >6, or (ci + ct) >3.

Following complete clinical and pathologic reviews, 140 biopsies (from 140 different patients) remained that met all study criteria. We were able to obtain complete follow-up information (initial response to anti-rejection therapy and subsequent graft survival for a minimum of 3 months post-biopsy or until graft failure) for 131 of these patients; the latter then comprised the study population used for analysis of treatment response and graft survival. One hundred two biopsies showed type 2A acute rejection and 29 type 2B acute rejection. All study procedures were approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Hospital.

Data analysis

For descriptive analyses categorical variables were divided into two or three groups of similar size, in as much as the data allowed. For multivariate analysis variables were dichotomized in order to help avoid over-parameterizing the statistical models.

To facilitate analysis of short-term responses of acute rejection episodes to therapy, treatments given were divided into three broad categories: (*1*) steroids only consisted of pulse intravenous methylprednisolone, with or without additional oral prednisone; (*2*) steroids plus consisted of the above plus an increase in the dose of tacrolimus or cyclosporine and/or addition of mycophenolate mofetil; and (*3*) antibody therapy consisted of OKT3, thymoglobulin, or Atgam, with or without additional change(s) in immunosuppressive therapy. All but four patients receiving antibody (3 with type 2A acute rejection, 1 with type 2B) also received steroids, and in most instances steroids and antibody were administered concurrently. Five patients (3 with type 2A rejection, 2 with type 2B) received antibody after failing to respond to a course of steroids; in these cases the response to therapy analyzed (see below) was that to the antibody.

The response of each rejection episode to anti-rejection therapy was graded according to criteria previously defined by Gaber et al [1]. A complete response to therapy occurred if the post-treatment serum creatinine returned to ≤125% of the pre-rejection baseline value or for cases of DGF, to ≤1.4 mg/dL. A complete response also was considered to have occurred if a repeat biopsy within 20 days of initiation of therapy showed no acute rejection (the latter defined as Banff 97 type 1A or greater) on an adequate sample with ≥10 glomeruli and ≥2 arteries [9]. A partial response occurred if there was a reduction in serum creatinine with therapy, but only to within 126 to 175% of the pre-rejection baseline value; for DGF cases recovery of serum creatinine to within the range of 1.5 to 2.9 mg/dL was considered a partial response. A partial response was also considered to have occurred if a repeat biopsy within 20 days of initiation of therapy showed a lower grade of acute rejection (Banff 97 type 1A or greater) than was present on the original biopsy, on an adequate sample as defined above.

Statistical analysis

Clinical and pathologic data, devoid of personal identifiers, were entered into an electronic database and the accuracy of data transcription was independently confirmed. The distribution of variables was examined using the Shapiro-Wilks test and q-q plots. Outlying values were identified using box plots and their accuracy checked against the original source data. Normally distributed continuous data were summarized using means and standard deviations (SD) and were compared across groups using independent sample *t* tests. Markedly skewed data were summarized as medians and intra-quartile ranges (IQR) and were compared across groups using the Mann-Whitney test. Categorical data were expressed as percentages and compared using Fisher's exact test for dichotomous variables or otherwise by the chi square test.

The association of the Banff v score (v1 versus v2) with short-term response to therapy was examined using unconditional logistic regression. To examine the independence of this association from the other Banff acute indices the v score was simultaneously adjusted for g, i and t scores using multivariate regression. To examine the potential influence of other variables the v score was adjusted for each variable for which data were available in separate analyses.

Long-term graft outcome was measured from the time of transplantation and was examined by time to event analysis using the Kaplan-Meier method and log rank testing. Patients who died with functioning grafts were censored at that time. The independence of v score (that is, type 2A vs. type 2B acute rejection) regarding graft survival was examined using Cox proportional hazards modeling. More extensive modeling was not attempted due to the limited available sample size. Routine model checking was performed using analysis of residuals for the logistic regression and visual inspection of log-log plots for the Cox model. All analyses were two-tailed and used a type I error rate of 0.05. Analyses were performed using SPSS Base 7.5 (SPSS Inc., Chicago, IL, USA).

Table 1. Baseline clinical characteristics and histological findings of 131 renal allograft recipients at first biopsy proven episode of Banff 97 type 2 rejection

Parameter	All subjects ($N = 131$)	Banff type		P
		2A ($N = 102$)	2B ($N = 29$)	
Baseline clinical data				
Age *years*, mean (SD)[a]	43.3 (15.9)	43.0 (15.6)	44.5 (17.0)	0.66
Gender				
Male	72 (55%)	53 (52%)	19 (65.5%)	
Female	59 (45%)	49 (48%)	10 (34.5%)	0.21
Transplant type				
Cadaveric	82 (62.6%)	60 (58.8%)	22 (75.9%)	
Living	49 (37.4%)	42 (41.2%)	7 (24.1%)	0.09
Time to biopsy *days*				
Median (IQR)	12 (28)	11 (24.5)	16 (38.5)	0.52
Biopsy indication				
Delayed graft function	58 (44.3%)	46 (45.1%)	12 (41.4%)	
Rise in serum creatinine	73 (55.7%)	56 (54.9%)	17 (58.6%)	0.72
Baseline immunosuppression				
with mycophenolate/rapamycin	81 (61.8%)	66 (64.7%)	15 (51.7%)	
without mycophenolate/rapamycin	50 (38.2%)	36 (35.3%)	14 (48.3%)	0.30
Treatment[b]				
Steroids only	41 (31.3%)	38 (37.3%)	3 (10.3%)	
Steroids plus	30 (22.9%)	23 (22.5%)	7 (24.1%)	
Antibody therapy	60 (45.8%)	41 (40.2%)	19 (65.5%)	0.02
Pathology data				
g score				
0	53 (40.5%)	42 (41.2%)	11 (37.9%)	
1	59 (45.0%)	49 (48.0%)	10 (34.5%)	
2–3	19 (14.5%)	11 (10.8%)	8 (27.6%)	0.07
i score				
0–1	23 (17.6%)	19 (18.6%)	4 (13.8%)	
2	35 (26.7%)	31 (30.4%)	4 (13.8%)	
3	73 (55.7%)	52 (51.0%)	21 (72.4%)	0.11
t score				
0–1	57 (43.5%)	45 (44.1%)	12 (41.4%)	
2	39 (29.8%)	27 (26.5%)	12 (41.4%)	
3	35 (26.7%)	30 (29.4%)	5 (17.2%)	0.23
Unmodified acute SUM score[c]				
1–5	47 (35.9%)	43 (42.2%)	4 (13.8%)	
6–7	50 (38.2%)	38 (37.3%)	12 (41.4%)	
8–10	34 (26.0%)	21 (20.6%)	13 (44.8%)	0.006
Chronic allograft nephropathy score				
0–1	60 (45.8%)	45 (44.1%)	15 (51.7%)	
2–4	43 (32.8%)	35 (34.3%)	8 (27.6%)	
5–6	28 (21.4%)	22 (21.6%)	6 (20.7%)	0.74
Edema score[d]				
0–1	27 (20.6%)	22 (21.6%)	5 (17.2%)	
2	43 (32.8%)	36 (35.3%)	7 (24.1%)	
3	61 (46.6%)	44 (43.1%)	17 (58.7%)	0.33
Acute tubular necrosis				
No	121 (92.4%)	94 (92.2%)	27 (93.1%)	
Yes	10 (7.6%)	8 (7.8%)	2 (6.9%)	0.87
Tacrolimus/cyclosporine toxicity				
No	98 (74.8%)	77 (75.5%)	21 (72.4%)	
Yes	33 (25.2%)	25 (24.5%)	8 (27.6%)	0.75

[a] Abbreviations are: SD, standard deviation; IQR, intraquartile range

[b] Definitions are: steroids only, pulse intravenous corticosteroid; steroid plus, pulse intravenous corticosteroid with increased tacrolimus/cyclosporine dose and/or the addition of mycophenolate; antibody therapy, antibody therapy with or without other interventions. (Details of therapy are in Fig. 2)

[c] Sum of individual Banff indices, (g + i + t + v)

[d] Estimated fraction of cortical tissue present with edema: 0, <10%; 1, 10–25%; 2, 26–50%; 3, >50%

RESULTS

Demographic data for the 131 patients with biopsies meeting study entry criteria and having complete data available for analysis are listed in the upper portion of Table 1. Fifty-five percent of the patients were male, 62.6% received a cadaveric graft (including 3 simultaneous kidney-pancreas grafts), 31.3% a living-related graft and 6.1% a living-unrelated graft. The median time from transplantation to biopsy was 12 days (range 3 to 2484 days). The indication for biopsy was DGF in 58 cases and a rise in serum creatinine in 73. The biopsy indication was strongly correlated with time post-transplantation,

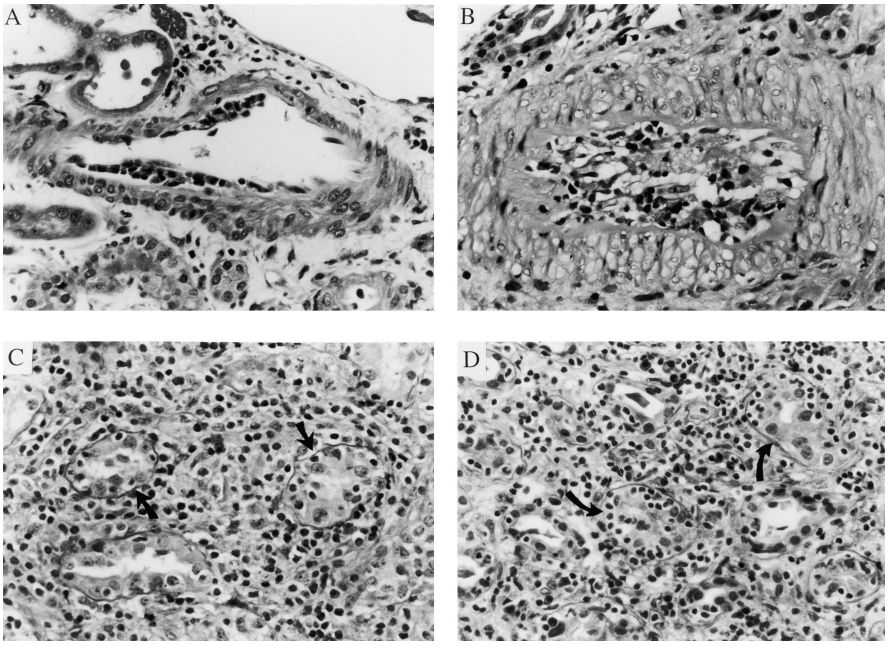

Fig. 1. Histopathologic lesions of acute rejection as defined by Banff 97 classification. (*A*) Mild to moderate intimal arteritis (Banff index v1); there are multiple mononuclear inflammatory cells directly beneath the endothelium but with <25% reduction in luminal area (H&E, ×400). (*B*) Severe intimal arteritis (v2), with subendothelial mononuclear cells, edema, and fibrin resulting in >25% reduction in luminal area (H&E, ×400). (*C*) Moderate tubulitis (t2); two tubular cross-sections (arrows) contain between 5 and 10 infiltrating mononuclear inflammatory cells. Note the accompanying interstitial inflammation (PAS, ×400). (*D*) Severe tubulitis (t3), with >10 infiltrating mononuclear inflammatory cells in multiple tubular cross sections (arrows). The surrounding interstitium is edematous with mononuclear cell infiltrate (PAS, ×400).

with DGF being the indication for 71% (27 of 38) of biopsies done during the first 7 days after the transplant, 51% (30 of 59) of biopsies done between days 8 and 30, and only 3% (1 of 34) of biopsies performed after day 30 ($P < 0.001$). Cases of type 2A versus 2B rejection did not differ significantly with respect to patient age, transplant type, mean time from transplant to biopsy, biopsy indication, or baseline immunosuppression (use of mycophenolate mofetil or rapamycin versus azathioprine or none of these three agents). However, cases of type 2B rejection were treated more aggressively: 19 (66%) of these 29 patients received antibody therapy (15 OKT3, 4 thymoglobulin) and only 3 (10%) corticosteroids alone. By contrast, 41 (40%) of the 102 patients with type 2A rejection received antibody therapy (32 OKT3, 6 thymoglobulin, 3 Atgam) and 38 (37%) received corticosteroids alone (Table 1).

The histopathologic lesions of acute rejection as defined by the Banff 97 classification [9] are illustrated in Figure 1. Figure 1 A and B show lesions of intimal arteritis, with mononuclear inflammatory cells beneath the endothelium, accompanied by edema fluid and fibrin in the artery shown in Figure 1B. In the latter artery this inflammatory process results in loss of >25% of the luminal area, which defines a lesion of severe intimal arteritis (Banff index v2) and is required for diagnosis of Banff 97 type 2B acute rejection. In the artery shown in Figure 1A, the degree of luminal obstruction is less than 25%, defining a lesion of mild to moderate intimal arteritis (Banff index v1) and a diagnosis of type 2A acute rejection. Figure 1 C and D illustrate moderate (Banff index t2) and severe (t3) tubulitis, with 5 to 10 and >10 mononuclear cells, respectively, in the most involved tubular cross-sections.

Of the 131 biopsies included in this study, 101 had been assigned a Banff acute rejection type (grade) at the time of original diagnosis, 25 by the pathologist who performed slide reviews for this study and 76 by another renal pathologist. In all 25 of the former cases and in 73 of the latter 76, the original and review diagnoses were in agreement, taking into account differences between the original (1993) Banff and Banff 97 schemata regarding typing of specific histologic lesions of acute rejection [9, 10]. Two biopsies originally diagnosed as showing type 2A acute rejection were believed to have type 2B rejection on review, and one case originally diagnosed as type 2B rejection was thought to show type 2A. Biopsies showing type 2A versus type 2B acute rejection did not differ significantly with respect to the mean number of tissue cores (2.0 ± 0.8 vs 1.9 ± 0.6, mean ± SD), or the mean (6.2 ± 3.2 vs. 6.4 ± 3.3) or median number of arteries (6, range 2 to 15 vs. 5, range 2 to 16) present per biopsy. However, the percentage of visualized arteries showing intimal arteritis (v1 or v2) was significantly higher in biopsies with type 2B rejection (median 36%, range 17 to 100%) than in those with type 2A (median 25%, range 7 to 67%; $P < 0.001$).

Histopathologic features of the 131 biopsies are summarized in the lower portion of Table 1. As noted above, 102 of the biopsies showed mild-to-moderate intimal arteritis (Banff 97 index v1, type 2A acute rejection) while 29 showed severe intimal arteritis (index v2, type 2B

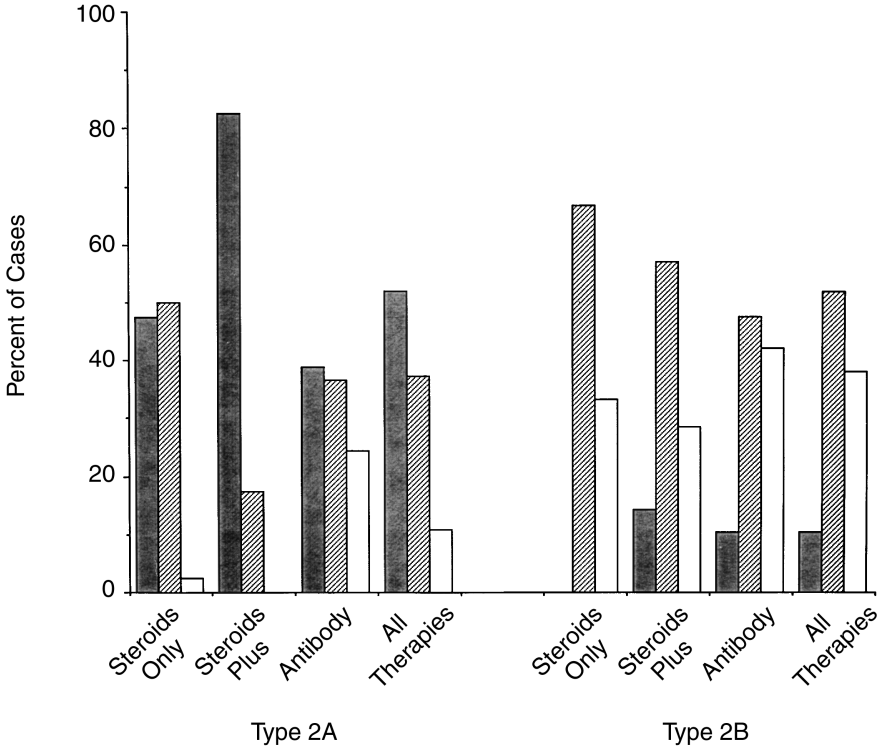

Fig. 2. Comparison of responses of types 2A and 2B acute rejection episodes to anti-rejection therapy. Criteria for complete and partial responses as well as therapeutic categories are defined in the Methods section; cases showing neither a complete or partial response are categorized as showing no response. Within each therapeutic category, the percent of cases with complete, partial, and no response to therapy are shown. Of the 60 patients receiving antibody, 47 received OKT3, 10 thymoglobulin, and 3 Atgam; all but 4 of the patients receiving antibody also received steroids. Additional therapies in the Steroids Plus patients included increased tacrolimus dosage (25 cases), increased tacrolimus plus mycophenolate mofetil (2), increased cyclosporine (1), increased cyclosporine plus mycophenolate mofetil (1) and mycophenolate mofetil (1). Symbols are: (■) complete response; (▨) partial response; (□) no response.

acute rejection). Banff acute SUM scores (g + i + t + v) were significantly higher in biopsies showing 2B lesions, in part because the v score is included within this SUM, although a modified acute SUM (g + i + t) without the v component was >5 in 86% of 2B lesions versus 58% of 2A ($P = 0.005$). There was also a trend toward more glomerulitis (g) and interstitial inflammation (i) in the 2B biopsies, although this was not statistically significant. There were no significant differences between biopsies with type 2A versus 2B acute rejection with respect to tubulitis (t), CAN score, edema score, the presence or absence of acute tubular injury (ATN, in areas of the biopsy not appearing to be directly involved by acute rejection) and histologic evidence of cyclosporine/tacrolimus nephrotoxicity.

Short-term outcome

By univariate analysis, there was a significant difference between type 2A (v1) and type 2B (v2) acute rejection episodes with regard to initial response to anti-rejection therapy: 52.0% versus 10.3% showed a complete response, 37.3% versus 51.7% a partial response, and 10.8% versus 37.9% no response, respectively (Fig. 2). This difference occurred despite the more aggressive treatment of type 2B rejection (Fig. 2; also see in prior section). As shown in Table 2, the odds ratio for failing to obtain a complete response to therapy was significantly associated not only with the v score, but also with the extent of tubulitis (absent or mild vs. moderate to severe) and the modified acute SUM score (g + i + t). Interstitial inflammation, glomerulitis, edema score, CAN score, presence or absence of ATN or calcineurin inhibitor toxicity, baseline immunosuppression and type of anti-rejection therapy given were not significantly associated with treatment response, although the response was significantly worse (that is, higher odds of partial/no response) in patients with DGF than in those biopsied for a rise in serum creatinine. As noted in Table 3, the v and t indices as well as indication for biopsy remained significant predictors of initial response to therapy in a multivariate analysis adjusting for each of the listed parameters. In addition, the v score remained significant when adjusted in separate analyses for the modified acute SUM score, CAN score, and other parameters listed in Table 2 (data not shown).

Long-term follow-up

The cohort provided a total of 290 patient-years of follow-up, 81% of which was provided by patients with type 2A acute rejection. The median follow-up interval post-biopsy for patients whose grafts continued to function at the end of the observation period was 20 months (range 3 to 157). As shown in Table 4, the incidence of graft failure was significantly higher with type 2B as compared with type 2A acute rejection. The mean duration of follow-up and median serum creatinine levels in surviving grafts were not significantly different between these two groups.

Table 2. Univariate associations of arteritis score, baseline clinical characteristics and other histological findings with partial/no response as compared with complete short-term response to therapy

	Odds ratio	95% CI[a]	*P*
Arteritis score			
v score			
v1	Ref		
v2	9.4	2.7, 32.9	<0.001
Baseline clinical characteristics			
Age *years*	1.01	0.99, 1.04	0.27
Gender			
Female	Ref		
Male	1.42	0.71, 2.85	0.33
Transplant type			
Living	Ref		
Cadaveric	2.0	0.96, 4.02	0.07
Duration transplantation-biopsy			
≤7 days	0.90	0.39, 2.05	0.79
8–30 days	Ref		
>30 days	1.49	0.63, 3.50	0.36
Biopsy indication			
Rise in serum creatinine	Ref		
Delayed graft function	2.77	1.33, 5.73	0.006
Baseline immunosuppression			
without mycophenylate/rapamycin	Ref		
with mycophenylate/rapamycin	0.95	0.47, 1.94	0.89
Treatment			
Steroids only	Ref		
Steroids plus	0.50	0.19, 1.31	0.16
Antibody	2.02	0.88, 4.60	0.10
Other histological findings			
g score			
0	Ref		
1–3	0.81	0.40, 1.64	0.55
i score			
0–2	Ref		
3	1.70	0.85, 3.42	0.14
t score			
0–1	Ref		
2–3	3.05	1.48, 6.27	0.002
Modified acute SUM score[b]			
1–5	Ref		
6–9	2.23	1.08, 4.61	0.03
Edema score[c]			
0–1	Ref		
2	1.34	0.54, 3.33	0.53
3	1.04	0.47, 2.29	0.93
Chronic allograft nephropathy score			
0–1	Ref		
2–6	1.19	0.59, 2.37	0.63
Acute tubular necrosis			
Yes	Ref		
No	2.13	0.57, 7.94	0.26
Tacrolimus/cyclosporine toxicity			
Yes	Ref		
No	0.98	0.44, 2.18	0.96

[a] Abbreviations: CI, confidence interval; Ref, reference category
[b] Sum of individual Banff indices, excluding v, (g + i + t)
[c] Estimated fraction of cortical tissue present with edema: 0, <10%; 1, 10–25%; 2, 26–50%; 3, >50%

Figure 3 shows unadjusted Kaplan-Meier graft survival curves for types 2A and 2B acute rejection. Median graft survival from the time of transplantation was 7.5 years (95% CI, 3.0 to 12.0) for type 2A versus 4.6 years (95% CI, 0.4 to 8.8) for type 2B ($P = 0.05$). Using the Cox proportional hazards model (Table 5), the relative hazard of graft failure with type 2B rejection (v2 lesion) as compared with type 2A (v1) was 1.9 ($P = 0.05$). The only other variables significantly associated with graft survival were response to therapy with a partial response and no response being associated with relative hazards of 3.3 and 22.8, respectively, compared with a complete response, the interval between transplantation and biopsy, and interstitial inflammation. In the latter instances, rejection episodes diagnosed more than 30 days post-transplantation were associated with a poorer graft survival

Table 3. Multivariate logistic regression of partial/no response as compared to complete response to treatment

	Adjusted odds ratio	95% CI[a]	*P*
v score			
v1	Ref		
v2	13.2	3.3, 52.7	<0.001
Biopsy indication			
Rise in serum creatinine	Ref		
Delayed graft function	6.34	2.34, 17.17	<0.001
t score			
0–1	Ref		
2–3	5.0	1.90, 13.20	0.001
i score			
0–2	Ref		
3	1.56	0.58, 4.18	0.38
g score			
0	Ref		
1–3	0.88	0.36, 2.12	0.77

[a] Abbreviations: CI, confidence interval; Ref, reference category

than earlier rejection episodes, and moderate interstitial inflammation (Banff 97 index i2) was associated with better graft survival than minimal or mild inflammation (i0 to i1). Notably, the extent of tubulitis was not significantly associated with graft survival. As shown in the right side of Table 5, the deleterious effect of v2 versus v1 lesions with regard to graft survival was modestly attenuated by adjusting for transplant type, acute glomerulitis (g) score and modified acute SUM score, but only marginally influenced by adjustment for the other various histologic and baseline clinical parameters examined. However, when adjusted for the short-term response to treatment the prognostic effect of the v score on graft survival was abolished.

DISCUSSION

The primary objective of this study was to determine if the distinction between types 2A and 2B acute rejection, as defined in the Banff 97 working classification of renal allograft pathology [9], is justified based on short- and long-term clinical outcomes. Although the clinical relevance of most aspects of the Banff schema has been tested in multiple studies and shown to have prognostic significance with regard to both reversibility of acute rejection episodes and long-term graft survival [1, 3, 5, 14], only very limited examination of the validity of subclassifying type 2 rejection on the basis on the degree of intimal arteritis present has been done. Our data show that patients with type 2B acute rejection have a much lower likelihood of showing a complete response to antirejection therapy than patients with type 2A acute rejection (10 vs. 52%), and a greater likelihood of having neither a complete or partial response to therapy (38 vs. 11%). The odds of graft failure are also higher in cases of type 2B acute rejection than in those of type 2A, although most or all of this difference in long-term prognosis is related to the initial response to therapy. Our findings are in agreement with those of Minervini et al, who found a greater incidence of graft loss among 18 patients with v2 intimal arteritis (type 2B acute rejection) than among 36 patients with v1 intimal arteritis (type 2A) [5]. Together, these studies appear to validate the distinction between types 2A and 2B acute rejection in the Banff 97 schema.

Another aim of this study was to determine if the short- and long-term outcomes of acute rejection with intimal arteritis are affected by the extent of interstitial inflammation and/or tubulitis present. In biopsies without arteritis these parameters, and especially tubulitis, define whether a biopsy shows acute rejection, and the type of rejection present (borderline, types 1A and 1B) according to the Banff 97 classification. In at least some studies, the type of acute rejection in these latter biopsies is a significant prognostic indicator of both response to anti-rejection therapy (particularly corticosteroids) and long-term graft function [3, 5, 14], and there is also some evidence suggesting that more extensive tubulointerstitial inflammation may adversely affect outcomes in acute rejection with intimal arteritis [6, 11]. In our 131 cases of types 2A and 2B acute rejection, the initial response to therapy was significantly better when the biopsy showed absent or mild tubulitis (fewer than 5 lymphocytes in the most involved tubular cross-section) than when moderate or severe tubulitis was present. However, long-term graft survival was not significantly associated with the degree of tubulitis present. Nickeleit et al found in 57 cases of acute rejection with intimal arteritis a trend toward increasing graft failure at one year post-biopsy when there was also a prominent degree of tubulitis ($\geq$7 non-atrophic tubular cross-sections with at least one invading mononuclear cell in 10 consecutive high power microscopic fields), although as in our study this did not reach statistical significance [6].

Consistent with findings of others [6, 15], we found no evidence that increasing interstitial inflammation worsened the short- or long-term prognosis in cases of acute rejection with intimal arteritis. Somewhat surprisingly, such rejection episodes with moderate interstitial inflammation (Banff index i2) had a better outcome than those with minimal or mild (i0 or i1) inflammation. Nickeleit et al also found a similar trend in their cases of type 2 acute rejection, with lesions having 11 to 25% cortical infiltrate (an amount corresponding to Banff index i1) being associated with a worse 1-year graft survival than those with more widespread interstitial infiltrate, although the number of cases in the former group was small and the difference was not statistically significant [6]. The reason(s) for this apparent "protective" effect of moderate interstitial inflammation are not clear, al-

Table 4. Renal allograft outcome among 131 recipients with biopsy proven Banff 97 type 2 acute rejection

	All subjects ($N = 131$)	Banff 97 Type		P
		2A ($N = 102$)	2B ($N = 29$)	
Short-term response to treatment				
No response, *N (%)*	22 (16.8%)	11 (10.8%)	11 (37.9%)	
Partial response, *N (%)*	53 (40.5%)	38 (37.3%)	15 (51.7%)	
Complete response, *N (%)*	56 (42.7%)	53 (52.0%)	3 (10.3%)	<0.001
Long-term graft survival				
Follow-up status, *N (%)*				
Functional	91 (69.5%)	75 (73.5%)	16 (55.2%)	0.07
Failed	40 (30.5%)	27 (26.5%)	13 (44.8%)	
Survival *years*, median (SE)	4.7 (1.2)	7.5 (2.3)	4.6 (2.2)	0.05
Incidence rate of graft failure, (failures per graft-year)	1 per 7.25	1 per 8.7	1 per 4.2	0.04
Serum creatinine at last follow-up, mg/dL, median (IQR)	1.65 (0.93)	1.60 (0.95)	1.80 (1.23)	0.36

Abbreviations are: SE, standard error; IQR, intraquartile range.

though it would appear unlikely to be related to the inclusion of significant numbers of cases with a component of antibody-mediated acute rejection in our study population. We specifically excluded patients with documented anti-donor antibodies; however, it is certainly possible that some cases of acute rejection with an undocumented humoral component were included, particularly from early in the study interval before histologic [16] and more recently immunohistologic [17] findings correlating with the presence of donor-specific antibodies (and thus prompting testing for such antibodies) were described. While biopsies of acute rejection with a humoral component ("accelerated" acute rejection) not infrequently show scant interstitial inflammation [18], Trpkov et al found that minimal to mild tubulitis (Banff indices t0 and t1), but not a specific degree of interstitial inflammation, correlated strongly with the presence of donor-specific antibodies in acute renal allograft rejection [16]. The finding that minimal-to-mild tubulitis also correlated with a good outcome (particularly short-term) in the present study argues against cases with antibody-mediated rejection explaining our findings regarding interstitial inflammation.

Gaber and coworkers [1] and others [19, 20] have advocated the use of the acute SUM score, comprised of Banff indices (g + i + t + v), in the evaluation of acute renal allograft rejection. In the series of Gaber et al the reversal of acute rejection with corticosteroids correlated with the acute SUM score and the vascular (v) score [1]. There was no significant correlation with individual g, i, or t scores. In cases of acute rejection with intimal arteritis, the present study similarly showed the v score to be an independent prognostic indicator of clinical outcomes, and a higher modified acute SUM score (without the v component; g + i + t) also correlated significantly with a poorer initial response to therapy. However, the modified acute SUM score did not have a statistically significant correlation with long-term graft survival.

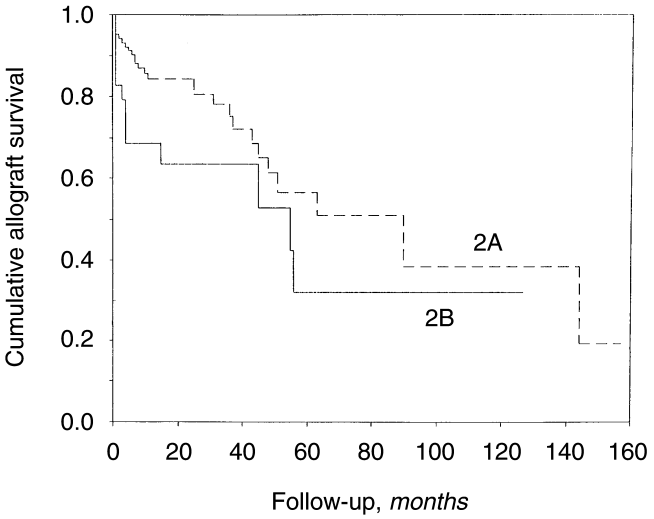

Fig. 3. Kaplan-Meier plots of unadjusted renal allograft survival from the time of transplantation among patients with Banff 97 type 2A ($N = 102$) and type 2B ($N = 29$) acute rejection. $P = 0.05$.

Biopsies with a chronic allograft nephropathy (CAN) score (Banff 97 indices cg + ci + ct + cv + ah + mm) >6, or with chronic indices (ci + ct) >3 were excluded from this study. These thresholds are somewhat arbitrary, but were chosen to exclude cases with more than mild CAN as it was the aim of this study to focus specifically on outcomes in acute rejection. It is well documented that CAN is an independent risk factor for the development of graft dysfunction [21–23]. In a study of patients who underwent protocol biopsies of renal allografts, the CAN score and (ci + ct) 6 months post-transplantation both correlated significantly with the rate of decline in serum creatinine over the ensuing 18 months [21]. In a similar study of protocol biopsies taken 6 months post-transplantation, grafts with >25% interstitial fibrosis (roughly equivalent to Banff ci index ≥2) had a significantly lower survival rate than those with <25% interstitial fibrosis (ci ≤1) [22]. In protocol biop-

Table 5. Association of crude and adjusted arteritis score and other unadjusted potential predictors on time to graft failure

				Arteritis score (v2 vs. v1)		
				Crude HR	95% CI	P^c
				1.9	0.99, 3.77	0.05
Potential explanatory variables	Crude HR[a]	95% CI	P	Adjusted HR[b]	95% CI	P^c
Age *years*	1.00	0.98, 1.03	0.68	1.93	0.99, 3.76	0.05
Gender						
Male	Ref					
Female	0.79	0.41, 1.49	0.46	1.89	0.97, 3.70	0.06
Transplant type						
Cadaveric	Ref					
Living	0.59	0.29, 1.18	0.13	1.76	0.89, 3.48	0.11
Duration transplant-biopsy						
≤7 days	1.63	0.66, 4.07	0.29			
8–30 days	Ref					
>30 days	2.61	1.27, 5.35	0.009	2.34	1.18, 4.65	0.02
Biopsy indication						
Delayed graft function	Ref					
Rise in serum creatinine	0.76	0.39, 1.47	0.41	1.89	0.96, 3.70	0.06
g score						
0	Ref					
1	0.81	0.40, 1.65	0.57			
2–3	1.58	0.67, 3.75	0.30	1.76	0.87, 3.56	0.12
i score						
0–1	Ref					
2	0.34	0.12, 0.94	0.04			
3	0.55	0.26, 1.17	0.12	2.04	1.00, 4.12	0.05
t score						
0–1	Ref					
2	1.28	0.62, 2.62	0.50			
3	1.39	0.62, 3.12	0.42	1.98	1.00, 3.89	0.05
Modified acute SUM score						
1–5	Ref					
6–9	1.17	0.60, 2.28	0.64	1.71	0.82, 3.59	0.16
CAN score						
0–1	Ref					
2–4	0.95	0.43, 2.1	0.91			
5–6	1.83	0.87, 3.87	0.11	1.97	1.00, 3.86	0.05
Acute tubular necrosis						
No	Ref					
Yes	0.65	0.16, 2.71	0.56	1.92	0.98, 3.74	0.06
Tacrolimus/CsA toxicity						
No	Ref					
Yes	0.65	0.29, 1.49	0.31	1.93	0.99, 3.77	0.05
Initial response to treatment						
Complete	Ref					
Partial	3.3	1.3, 8.70	0.01			
None	22.83	8.85, 58.91	<0.001	0.58	0.27, 1.25	0.17
Treatment						
Steroids only	Ref			1.87	0.94, 3.69	0.07
Steroids plus	0.76	0.29, 2.02	0.58			
Antibody	1.16	0.58, 2.33	0.67			

[a] Abbreviations: HR, hazard ratio; CI, confidence interval; Ref, reference category; CsA, cyclosporine
[b] Adjusted hazard ratio for arteritis score adjusted individually in separate analyses for the adjacent potential explanatory variable
[c] P value for comparison of time to graft failure for v2 vs. v1, either unadjusted (first row) or adjusted separately for each listed variable

sies taken two years post-transplantation, it was found that a six-component "chronic allograft damage index," in which five of the components are identical to those in the CAN score, was a significant and independent predictor of deterioration in graft function over the ensuing two years [23].

Among clinical parameters, we found that cases of type 2 acute rejection associated with DGF had a worse short-term clinical outcome compared with those of an initially functioning graft presenting with a rise in serum creatinine. It remains debated whether DGF correlates with reduced graft survival independent of acute rejection [24]. Still, in the context of our findings it is of interest that Marcén et al reported that the negative impact of acute rejection in the first month post-transplantation on one- and six-year graft survival rates was

significantly greater in patients with DGF than in those without DGF [24]. We also found that rejection episodes diagnosed more than 30 days post-transplantation were associated with a poorer graft survival than those diagnosed earlier in the post-transplant course. This is consistent with several previous studies that found that late acute rejection episodes (defined in these studies as >60 days post-transplantation) were associated with more frequent development of chronic rejection/CAN and graft loss due to chronic rejection than earlier episodes of acute rejection [25–27].

Our study is limited by insufficient data regarding a variety of factors known to influence long-term graft survival. However, this does not lessen the prognostic value of the Banff 97 distinction between types 2A and 2B acute rejection as demonstrated in this study. Our moderate sample size prevented us from conducting more extensive regression modeling, and in this regard a multicenter collaborative approach (such as [2]) is likely to be required to reach this goal. Alternatively, the fact that our study was based on experience in a single institution helps to limit variability in the management of acute rejection over the course of the study. An additional strength of this study is that all biopsies were reviewed and graded according to Banff 97 criteria by a single experienced renal pathologist while blinded to clinical data.

In summary, the findings of this study strongly support the distinction of types 2A and 2B acute rejection as defined in the Banff 97 working classification of renal allograft pathology. We also found that among lesions of acute rejection with intimal arteritis, a moderate to severe degree of tubulitis and a high modified acute SUM score (g + i + t) were associated with lower odds of a complete response to anti-rejection therapy. As such, it is our recommendation that pathology reports of biopsies showing Banff 97 type 2A or 2B acute rejection clearly specify the extent of tubulitis, as well as the presence of a high (for example, ≥6) modified acute SUM score.

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