Focal peritubular capillary C4d deposition in acute rejection

Alexander B. Magil¹ and Kathryn J. Tinckam²

¹Department of Pathology and Laboratory Medicine and ²Division of Nephrology, Department of Medicine, St Paul’s Hospital, Vancouver, BC, Canada

Abstract

Background. Diffuse peritubular capillary (PTC) C4d deposition has been shown to be associated with relatively poor graft outcome. The significance of focal PTC C4d staining in the early post-transplant period is uncertain.

Methods. Sixty-five biopsies from 53 patients with acute rejection were graded (Banff ’97 criteria), stained for C4d, monocytes and T cells, and divided into three groups according to PTC C4d: (i) focal C4d (F) (14 biopsies, 14 patients), (ii) diffuse C4d (D) (23 biopsies, 15 patients) and (iii) no C4d (N) (28 biopsies, 24 patients). The three groups were compared with respect to a variety of biopsy and clinical parameters including outcome.

Results. The incidence of transplant glomerulitis and glomerular monocyte infiltration were significantly greater in F (64% and 2.0±2.0) and D (57% and 3.4±2.0) than in N (11% and 0.2±0.2). A significantly higher proportion of F (93%) demonstrated acute cellular rejection (Banff ’97 grade I/C21) than did D (35%). The F and D groups included significantly more females (50 and 67%, respectively) than did N (21%). The percentage of patients with a second or third transplant was higher in F (29%) and D (40%) than in N (8%) (P=0.0589). The proportion of patients with glomerular filtration rate <30 ml/min at 12, 24 and 48 months was higher in the D and F groups than in the N, and there was a statistically significant increasing trend in odds of this outcome occurring at 48 months across the three groups (D>F>N group) (P=0.0416).

Conclusion. The results suggest that the biopsy findings and clinical course in patients with focal PTC C4d staining are similar to those associated with diffuse C4d.

Keywords: acute rejection; focal c4d; monocytes; prognosis; transplant glomerulitis

Introduction

Acute humoral rejection (AHR) has been the subject of considerable interest in recent years. It has been shown to carry a relatively poor prognosis [1-4] and is often resistant to conventional anti-rejection therapy [5-8]. As unconventional measures such as plasma exchange, immunoabsorption, intravenous immunoglobulin (IVIG) and anti-CD20 monoclonal antibody may be of some benefit in AHR [6,9-12], recognition of this form of rejection in allograft biopsies is important.

Peritubular capillary (PTC) C4d deposition in renal allografts has been suggested as a marker of humoral rejection [13] and strong, diffuse (involving >50% of PTC) staining of PTC for C4d has been included as one of the criteria for the diagnosis of AHR in a recent update of the Banff ’97 classification of renal allograft rejection [14]. However, the significance of focal PTC C4d deposition is controversial [13]. In some studies, patients with diffuse or focal PTC C4d deposition have been grouped together [3,15-17] while in others (from one group), only patients with diffuse PTC C4d deposition were considered as being C4d positive [1,6-8]. Two previous studies comparing patients with focal PTC C4d to those with diffuse PTC C4d, have not noted any significant differences between them with respect to histological biopsy findings [17] and graft survival [15]. However, in a recent report graft loss occurred more frequently in patients with diffuse PTC C4d compared to those with focal C4d [18].

Some studies have demonstrated a significant association between neutrophilic and monocytic infiltration of allografts and diffuse PTC C4d deposition [1,19]. Other lesions that also appear to be correlated with diffuse PTC C4d deposition include arterial and glomerular fibrinoid necrosis and glomerulitis [1,19]. Because it is unknown whether the changes that correlate with diffuse PTC C4d deposition are also associated with focal PTC C4d, patients whose biopsies showed focal C4d were compared to those with diffuse C4d with respect to a variety of parameters.
Materials and methods

Patients

For the purposes of this study, only those patients who were biopsied within 6 months of transplantation and whose allograft biopsies demonstrated either diffuse (positive reaction in >50% of PTC) strong or focal (positive reaction in <50% of PTC and in more than 10 PTC per biopsy [20]) moderately strong or strong PTC staining for C4d (Figure 1) and histological evidence of acute tubular injury were included in the test groups. Twenty-three biopsies from 15 patients (10 females, five males) biopsied serially between 1 January 1999 and 30 June 2002 had a diffuse PTC C4d reaction (D group) and 14 biopsies from 14 patients (seven females, seven males) biopsied during the same period showed focal PTC C4d staining (F group). Twenty-eight serial biopsies, done between 1 January 1999 and 31 December 1999 from an unselected group of 24 patients (5 females, 19 males) with acute rejection by the Banff '97 criteria (at least grade 1A) [21] and no staining for PTC C4d, served as controls (N group). The demographic features of the N group were very similar to those of all C4d negative patients with acute rejection biopsied between 1 January 1999 and 30 June 2002. All biopsies were obtained prior to treatment. Patient and donor data were compiled primarily from the British Columbia Transplant Society renal transplant database. Chart reviews were performed when specific information was not available from the database.

Histology

Renal biopsies were divided into three portions for light microscopy, electron microscopy and immunohistochemistry. For light microscopy, tissue was fixed in either B5 fixative (63 biopsies) and embedded in paraffin or Karnovsky's fixative (two biopsies) and embedded in polyglycol methacrylate. Sections embedded in paraffin were cut at 2 µm and stained with haematoxylin-eosin (H&E), periodic acid-Schiff and periodic acid-silver methenamine. Sections embedded in polyglycol methacrylate were cut at 1 µm and stained with haematoxylin-eosin and periodic acid-silver methenamine.

Immunohistochemistry

The avidin–biotin 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). Snap-frozen sections from biopsies of membranous glomerulonephritis which show strong glomerular staining for C4d served as positive controls for C4d. Lymph node tissue was used as positive controls for CD68 and CD3. B5-fixed paraffin-embedded sections were stained with commercially available mouse monoclonal antibodies specific for CD68 (a marker for monocytes) and CD3 (T cell marker) (Dako, Carpinteria, CA). For the two biopsies in which the histological portion had been embedded in polyglycol methacrylate, acetone-fixed sections of snap-frozen renal tissue were stained for CD68 and CD3 with the above monoclonal antibodies (Dako, Carpinteria, CA). 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.

Clinical monitoring

The patients' serum panel reactive antibody (PRA) level was determined by standard lymphocytotoxic assay prior to transplantation. Flow cytometric crossmatch (FACSCalibur G3 Flow Cytometer, Becton-Dickenson) was performed at the time of transplantation in patients at high risk.
Initial immunosuppression was achieved using a standard triple therapy regimen of methylprednisolone, 1 mg/kg/day for 3–5 days then prednisone (0.7–1 mg/kg/day tapering to 0.3 mg/kg/day by 2 weeks post-transplant and 0.15 mg/kg/day by 6 months) (all patients), cyclosporine, 9.0 mg/kg/day adjusted for trough levels 425–500 µg/ml for the first 30 days then tapered gradually to levels of 100–150 µg/l for long-term maintenance (29 patients) or tacrolimus, 0.12–0.15 mg/kg/day adjusted for trough levels 10–15 µg/ml for the first 30 days then tapered gradually to levels of 5–8 µg/ml for long-term maintenance (11 patients) and mycophenolate mofetil, 2000 mg/day (all patients). Induction therapy was used in four patients at high risk (PRA > 30%) and consisted of a 7–10 day course of treatment with antilymphocyte antibodies (OKT3, Ortho Biotech, Raritan, NJ), 5 mg/day. All but one of the rejection episodes were treated with methylprednisolone for 3–6 days (total dose range 1500–4000 mg). Patients with Banff Grade IIA or greater rejections were treated with OKT3 for 7–10 days (5 mg/day). One C4dþ patient had plasma exchange alone for one rejection episode and methylprednisolone, plasma exchange and IVIG following the plasma exchange with a total of 10 exchanges for each episode. Another C4dþ patient received IVIG in addition to methylprednisolone and OKT3 for a rejection episode.

For the purposes of this study the end point for outcome at 12, 24 and 48 months was defined as glomerular filtration rate <30 ml/min (including graft failure) as estimated by the modified diet in renal disease (MDRD) Formula [22], which has validity in transplant recipients [23]. This particular threshold was chosen based on the current K-DOQI classification of chronic kidney disease (CKD) [24] 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 co-morbidities is high.

Quantitative analysis

All biopsies were scored according to the Banff '97 criteria [20] to determine the type and grade of the rejection reaction. Neutrophils (PMN), monocytes (MO) and T cells were counted in all glomeruli in each biopsy and expressed as the number of cells per glomerulus. The number of cortical PTC PMN and 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 mm²) in each biopsy was determined by counting the number of cortical PTC PMN, CI MO and cortical hpf’s and dividing the number of cells by the number of cortical hpf’s. For the quantitative analyses, only those biopsies with cortex containing a minimum of four glomeruli were used. Thus, two D biopsies and 3 F ones were not included in the MO and T cell count determinations.

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation (SD). Continuous variables were compared using the ANOVA and Tukey’s test and categorical variables were compared using the Chi square (χ²) test or Fisher’s exact test, where appropriate. A P-value < 0.05 for two sided univariate tests was considered significant. The Mantel–Haenszel test for linear trend was applied across C4d groups (none, focal, diffuse) for outcomes at 12, 24 and 48 months.

Results

Renal biopsy findings

All but two of the 23 biopsies from the 15 patients with strong diffuse PTC C4d staining (D group) contained more than 10 glomeruli. The other two biopsies had nine glomeruli each. All of the D biopsies had at least two interlobular arteries. Ten of the 14 biopsies from the F group had 10 or more glomeruli. The other F biopsies contained eight (two biopsies) and seven and nine (one biopsy each) glomeruli. Ten of the F biopsies contained two or more interlobular arteries, three had one interlobular artery while one had no interlobular arteries. Twenty of the 28 biopsies with no PTC C4d staining (N group) contained 10 or more glomeruli while eight had nine glomeruli. Two of the N biopsies lacked interlobular arteries. The mean intervals between the times of transplantation and times of biopsy for the D, F and N groups were 22 ± 20, 22 ± 26 and 31 ± 43 days, respectively (not significant).

The C4dþ biopsies can be divided into two subgroups based on whether there was a significant cellular component to the acute rejection reaction or not. Fifteen of the D biopsies (65%) demonstrated either mild interstitial mononuclear cellular infiltration (Banff ’97 score: i = 1) with either no, mild or moderate tubulitis (Banff ’97 score: t = 0, 1 or 2) or moderate interstitial inflammation (Banff ’97 score: i = 2) with no or mild tubulitis (Banff ’97 score: t = 0 or 1) and were graded as suspicious for acute cellular rejection (ACR) according to the Banff ’97 grading system [22]. Eight of the D biopsies (35%) had ACR (Banff ’97 grade IA (n = 5) or 2A (n = 3)). Only one of the F biopsies (7%) lacked a significant interstitial mononuclear cell component (Banff ’97 grade suspicious: score i = 1, t = 2). The other F biopsies (93%) showed ACR (Banff ’97 grades 1A (n = 2), 1B (n = 3), 2A (n = 7), 3 (n = 1)). The N biopsies all demonstrated significant ACR (Banff ’97 grade 1A (n = 13), 1B (n = 5), 2A (n = 9), 3 (n = 1)). Comparison of the three groups simultaneously with respect to proportion with significant cellular rejection (Banff ’97 grade 1 or more) demonstrated a significant difference (χ² = 31.8412, P < 0.0001). The difference between the F and D groups was significant (χ² = 9.7097, P = 0.0018). Finally, one patient had two biopsies, one of which showed focal PTC C4d staining and a subsequent one, done 8 days later, diffuse PTC C4d reaction.

The significant biopsy findings are summarized in Table 1. Compared to the N group, the F and D groups showed significantly more transplant glomerulitis (P = 0.0003), glomerular MO infiltration (at least one glomerulus per biopsy containing a minimum of one intracapillary CD68+ cell) (P < 0.0001), and acute tubular injury (characterized by tubular dilatation, increased basophilia of tubular epithelial cytoplasm,
tubular nuclear enlargement and hyperchromaticity and tubular nuclear mitoses) not associated with significant tubulitis ($t/2^1$) or inflammation ($i/2^1$) ($P < 0.0001$). There were proportionately more biopsies in the F and D groups with mean glomerular MO:T cell ratio $>1.0$ than in the N group ($P < 0.0001$). Neutrophilic tubulitis (infiltration of tubular epithelium by predominantly PMNs) was greater in the D group than in the other two groups ($P = 0.05$). Varying numbers of PMN were observed in the glomeruli and CI PTC in all of the D and most of the F and N biopsies. Cortical interstitial MOs were present in all biopsies but appeared more prominent in the F and D ones. In most of the immunostained sections, it could not be determined with confidence whether the CI CD68+ cells were within or outside the PTC. Most of the F (86%), D (78%) and N (79%) biopsies showed varying numbers of glomerular T cells. Intimal arterial fibrinoid necrosis was observed in one F and one N biopsy. None of the biopsies showed glomerular fibrinoid change. One D and one N biopsy demonstrated focal glomerular capillary thrombosis. Focal interstitial medullary haemorrhage was noted in four (22%) of the 18 D biopsies and in one (14%) of the 7 F biopsies but in none of the 18 N biopsies in which the medulla was present. The PTC dilatation (arbitrarily defined 10% or more of PTCs showing a cross-sectional diameter equal to at least three times the diameter of a lymphocyte nucleus) was observed more extensively and in proportionately more D (91%) and F (64%) biopsies than in N ones (50%) ($\chi^2 = 8.452, P = 0.0146$). None of the biopsies demonstrated the evidence of calcineurin inhibitor toxicity histologically. There was no evidence of significant chronic changes in any of the biopsies.

Glomerular staining for C4d tended to be relatively weak and predominantly mesangial in location in the N biopsies. The D biopsies showed strong capillary and mesangial staining for C4d. The F biopsies demonstrated intermediate strength glomerular reactions for C4d that were capillary and mesangial in location.

### Table 1. Comparison of focal C4d (F) to diffuse C4d (D) and negative C4d (N) groups with respect to biopsy findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>D</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n with glomerulitis (%)</td>
<td>9 (64)</td>
<td>13 (57)</td>
<td>3 (11)</td>
<td>0.0003b</td>
</tr>
<tr>
<td>n with glomerular monocytes (MOd) (%)</td>
<td>14 (100)</td>
<td>22 (96)</td>
<td>12 (43)</td>
<td>&lt;0.0001c</td>
</tr>
<tr>
<td>n with acute tubular injury (%)</td>
<td>3 (100)</td>
<td>22 (96)</td>
<td>11 (39)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>n with glomerular MO:T cell ratio &gt;1.0 (%)</td>
<td>9 (64)</td>
<td>18 (78)</td>
<td>3 (11)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>n with neutrophilic tubulitis (%)</td>
<td>1 (7)</td>
<td>8 (35)</td>
<td>3 (11)</td>
<td>0.05c</td>
</tr>
<tr>
<td>n with glomerular neutrophils (%)</td>
<td>14 (100)</td>
<td>22 (96)</td>
<td>26 (93)</td>
<td>NS</td>
</tr>
<tr>
<td>n with peritubular capillary neutrophils (%)</td>
<td>14 (100)</td>
<td>23 (100)</td>
<td>28 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>n with glomerular T cells (%)</td>
<td>12 (86)</td>
<td>18 (78)</td>
<td>23 (82)</td>
<td>NS</td>
</tr>
<tr>
<td>n with peritubular capillary dilatation (%)</td>
<td>9 (64)</td>
<td>21 (91)</td>
<td>14 (50)</td>
<td>0.005c</td>
</tr>
</tbody>
</table>

NS is not significant.

* a is number of biopsies.
* F vs D vs N, $\chi^2$ test.
* F vs D vs N, Fisher’s exact test.
* MO is monocyte.

### Table 2. Quantitative analysis of biopsy findings for the focal C4d (F), diffuse C4d (D) and negative C4d (N) groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>F</th>
<th>D</th>
<th>N</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes/glomerulus (range)</td>
<td>2.0±2.0 (0.2–6.6)</td>
<td>3.4±2.0 (0.1–8.4)</td>
<td>0.2±0.2 (0–1.0)</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Neutrophils/glomerulus (range)</td>
<td>0.5±0.4 (0.1–1.4)</td>
<td>0.8±0.6 (0–3.1)</td>
<td>0.3±0.3 (0–1.4)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>T cells/glomerulus (range)</td>
<td>1.3±1.4 (0–5.3)</td>
<td>1.4±0.9 (0–3.0)</td>
<td>1.1±1.7 (0–6.9)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerular MO:T cell ratio (range)</td>
<td>2.6±3.5 (0.27–12.5)</td>
<td>3.2±3.7 (0.1–14.0)</td>
<td>0.4±0.6 (0–2.2)</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Peritubular capillary neutrophils/hpf (range)</td>
<td>0.4±0.3 (0.1–0.8)</td>
<td>0.9±0.8 (0.1–3.5)</td>
<td>0.4±0.3 (0.1–1.1)</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Cortical interstitial monocytes/hpf (range)</td>
<td>11.7±8.1 (2.3–31.0)</td>
<td>12.9±9.2 (7.0–36.2)</td>
<td>6.5±5.0 (1.0–22.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS is not significant.

* F vs D, ANOVA and Tukey’s test.
* F vs N, ANOVA and Tukey’s test.
* MO is monocyte.

Quantitative analysis

The results are summarized in Table 2. The extent of glomerular infiltration by MOs as expressed by the mean number of MOs per glomerulus in the F group (2.0±2.0) was significantly less than that of the D group (3.4±2.0) ($P < 0.05$) but significantly greater than that of the N group (0.2±0.2) ($P < 0.05$).
The mean glomerular MO:T cell ratio in the F group (2.6 ± 3.5) was less than that of the D group (3.2 ± 3.7) (the difference was not significant) but was significantly greater than that of the N group (0.4 ± 0.6) \( (P < 0.05) \). The mean PMN/glomerulus in the F group (0.5 ± 0.4) was less than that of the D group (0.8 ± 0.6) but higher than that of the N group (0.3 ± 0.3), the differences not being significant. The mean number of PTC PMNs per high power field was significantly higher in the D group (0.9 ± 0.8) than in the F or N ones (0.4 ± 0.3) \( (P < 0.05) \). The CI MO infiltration (mean CI MO/hpf) was greater in the F group (11.7 ± 8.1) than in the N group (6.5 ± 5.0), but less than in the D group (12.9 ± 9.2), the differences not being significant. The level of glomerular T cell infiltration was very similar in the three groups.

### Clinical data

There were proportionately more females in the F (50%) and D (67%) groups than in the N group (21%) \( (\chi^2 = 8.557, P = 0.0139) \). The mean age of the recipients was similar in the three groups (D: 45.9 ± 12.3 years; F: 45.3 ± 10.8 years; N: 48.5 ± 10.8 years). The proportion of patients receiving a cadaveric transplant was similar in the three groups (D = 53%, F = 43% and N = 46%). Proportionately more D and F patients had a second or third transplant (40% and 29%, respectively) than did the N patients (8%) \( (\chi^2 = 5.664, P = 0.0589) \). The D group had a significantly higher proportion (62%) of patients with a PRA > 20% prior to transplantation than did the F (15%) or N (12%) groups \( (\chi^2 = 12.169, P = 0.0023) \). An anti-donor antibody determination at the time of rejection was performed in only one patient and this was positive. The severity of the rejection reactions expressed as the change in serum creatinine \( \Delta Cr \) was 63 ± 59, 86 ± 74 and 121 ± 115 μmol/l for the F, D and N groups, respectively, the differences not being significant. Monoclonal anti-lymphocyte antibody therapy was used in 53, 21 and 29% of the D, F and N patients, respectively. One of the D patients was treated for two rejection episodes by plasma exchange (with IVIG for the second episode) with full response. The other D patient, treated with IVIG alone, did not respond. Although, the final Cr, one month after biopsy, was higher in the D group (226 ± 163 μmol/l) than in the F (173 ± 60 μmol/l) or N (179 ± 57 μmol/l) groups, the differences were not significant.

For the outcome analyses, the patient with focal C4d on her first biopsy and diffuse C4d on the second biopsy was considered to be part of the F group. One D patient died during the first post-transplant year and is omitted from the analysis. Table 3 presents the results of the outcome analysis at 12, 24 and 48 months post-transplant. There were no significant differences in outcome at any of the three time intervals. However, there was a statistically significant trend in the increase in odds of the outcome \( \text{MDRD} < 30 \text{ml/min} \) occurring at 48 months in the three groups (D > F > N group) \( (P = 0.0416) \) (Table 4).

![Discussion](https://academic.oup.com/ndt/article-abstract/21/5/1382/1822112/2151219212@2151219212)

\[ \]
compared to the focal C4d group [18]. While the results of the present study are generally similar with respect to histology and outcome, there are some differences. There was a much higher incidence of transplant glomerulitis (D = 64%, F = 57%) in the present investigation than in the previous one (D = 26%, F = 0%) [18]. Diffuse C4d was associated with a higher rate of graft failure in the previous study [18], whereas in the present one, graft outcome was similar although there was a significant trend in the increase in odds of graft failure occurring at 48 months with the diffuse C4d group having a greater odds ratio at 48 months than the focal group. The difference in the timing of the acute rejection reactions (early vs late) may account, at least in part, for the differing results.

Some investigations have shown a strong correlation between diffuse PTC C4d deposition and either circulating anti-donor specific antibodies [1,4] or anti-ABO blood group antibodies in ABO-incompatible renal transplantation [24] suggesting that diffuse PTC C4d staining in biopsies can serve as a tissue marker for AHR [25]. Whether the same is true for focal PTC C4d deposition is uncertain. The previous studies that did examine patients with focal C4d separately did not present data on circulating anti-donor specific antibodies [15,18]. Unfortunately, in the present retrospective study, anti-donor specific antibodies were not searched for in the patients with focal PTC C4d at the time of biopsy nor was the serum from that time saved precluding later testing. However, we have encountered three allograft patients very recently (not included in this study because of very short follow-up) with focal PTC C4d for whom there was testing for circulating anti-donor specific antibodies (flow cross match). Two patients had circulating anti-donor specific anti-HLA II antibodies (DR4 and DR7) and one was negative (data not shown).

In a recent study, MO infiltration, both glomerular and interstitial, but especially glomerular, was closely correlated with diffuse PTC C4d deposition [19]. Significant MO infiltration has also been observed in cardiac biopsies in heart transplant patients with AHR [26]. In the present investigation, biopsies with focal PTC C4d staining showed glomerular and CI MO numbers comparable to those seen in the diffuse C4d positive biopsies and greater than those in the C4d negative biopsies.

Another similarity between the focal and diffuse C4d positive biopsies was the finding of transplant glomerulitis in the majority of the biopsies from both of these groups in comparison to the low frequency of this phenomenon in C4d negative acute rejection. Several previous studies have noted the association of glomerulitis with AHR [27] and PTC C4d deposition in biopsies [17,19]. In one of these latter studies both focal and diffuse PTC C4d staining was considered positive for C4d [17] while in the other, only biopsies with diffuse C4d reactions were studied [19].

In a recent study of transplant glomerulitis, it was demonstrated that, in biopsies with diffuse PTC C4d deposition, MO were the predominant cells infiltrating the glomeruli, whereas in C4d negative biopsies with acute rejection and transplant glomerulitis, the predominant cell infiltrating the glomeruli was the T cell [28]. The results of the present study in which the predominant infiltrating cell in the glomeruli of the focal C4d positive biopsies was the MO further underline the similarities between the focal and diffuse C4d positive groups.

Glomerular and PTC neutrophilic infiltration have been shown to be associated with AHR [18] and diffuse PTC C4d deposition [1,7,19] and have been suggested as tissue markers suggesting AHR [14,29]. In the present study, there was a trend to increased neutrophils in glomeruli in the focal C4d positive group but this was not pronounced and not significantly different from that in the C4d negative biopsies. Although the correlation of neutrophilic infiltration with PTC C4d deposition or AHR has been emphasized in some studies [1,7,18], this association was weaker than that of MO infiltration with diffuse PTC C4d staining in an investigation reported recently by the authors [19]. Indeed, two other studies were unable to demonstrate a significant association between PTC C4d deposition and neutrophilic infiltration [7,17].

One major difference between the focal and diffuse C4d positive groups was the incidence of accompanying significant interstitial cellular rejection (at least Banff ‘97 grade 1A) which was significantly higher in the focal C4d positive group than in the diffuse C4d group. In several previous reports, the proportion of C4d positive biopsies having significant interstitial cellular rejection was less than 50% [3,15,17]. Of interest in this regard, in one investigation, which did present some results for both focal and diffuse C4d staining, the incidence of concurrent interstitial cellular rejection was higher in the focal C4d group [15]. In the other studies mentioned above [3,17], in one the C4d positive group contained both focal and diffuse C4d positive biopsies [17], while in the other it was not mentioned whether the C4d positive biopsies were focal or diffuse [3]. In contrast, interstitial cellular rejection was observed in the majority of biopsies exhibiting diffuse C4d positivity in one study [1]. Although the design of the present study does not allow one to determine the reason(s) for this difference, it is possible that the process resulting in focal C4d deposition was not clinically severe enough on its own to result in a decision to biopsy and that an additional insult in the form of cellular rejection was necessary to cause sufficient injury to warrant a biopsy.

Finally, in a recent study involving a larger cohort of patients from our laboratory, both glomerular MO infiltration and PTC C4d deposition were shown to have a significant adverse impact on long-term graft survival [30]. The trend of increasing odds of an adverse outcome at 48 months in the C4d negative, focal C4d positive and diffuse C4d positive groups with the latter two having significantly higher glomerular MO counts is consistent with the results of the above investigation.
References


30. Tinckam KJ, Djurdjev O, Magil AB. Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status. Kidney Int (accepted for publication)

Conflict of interest statement. None declared.