Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: informahealthcare.com/journals/irnf20

C4d deposition is associated with immune cells infiltrating in kidney allograft glomerulitis and peritubular capillaritis

Juan Jin, Yi Wen Li & Qiang He

To cite this article: Juan Jin, Yi Wen Li & Qiang He (2015) C4d deposition is associated with immune cells infiltrating in kidney allograft glomerulitis and peritubular capillaritis, Renal Failure, 37:5, 791-797, DOI: 10.3109/0886022X.2015.1033368

To link to this article: https://doi.org/10.3109/0886022X.2015.1033368

Published online: 14 Apr 2015.

RENAL FAILURE

http://informahealthcare.com/rnf
ISSN: 0886-022X (print), 1525-6049 (electronic)

Ren Fail, 2015; 37(5): 791–797
 DOI: 10.3109/0886022X.2015.1033368

informa
healthcare

CLINICAL STUDY

C4d deposition is associated with immune cells infiltrating in kidney allograft glomerulitis and peritubular capillaritis

Juan Jin, Yi Wen Li, and Qiang He

Department of Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, P.R. China

Abstract

Objective: The aim of our study was to determine the amount and composition of immune cells within glomeruli and PTCs and its relationship with C4d deposition. *Materials and methods*: Immunohistochemistry staining for C4d, CD3, CD68, granzyme B and Foxp3 was used for phenotyping and enumerating immune cells within intracapillaries. *Results*: C4d staining was present in 26 biopsy specimens ($C4d^+$) and negative in 25 specimens ($C4d^-$). The total number of infiltrating cells in glomerulus and PTC in $C4d^+$ was significantly higher than in $C4d^-$. Although the $C4d^+$ showed a significantly higher mean number of macrophages per glomerulus and PTC than in $C4d^-$ group, the $C4d^-$ showed a higher mean number of T cells per glomerulus and PTC than in $C4d^+$. Comparing cell counts in diffuse $C4d^+$ and focal $C4d^+$ groups, a significant difference of absolute numbers of intracapillary cells could be observed in glomeruli and PTCs. The mean number of macrophages per glomerulus and PTC in diffuse $C4d^+$ was greater than that of the focal $C4d^+$, while mean T cells per glomerulus and PTC were less in cases of diffuse $C4d^+$ than in focal $C4d^+$. The differences, however, did not achieve statistical significance. Not only glomerular T cells but also PTCs are granzyme B positive T cells totally. *Conclusion*: The total number of infiltrating cells in glomeruli and PTC has association with PTC C4d deposition; the infiltrating cells were predominantly macrophages in $C4d^+$, especially in diffuse $C4d^+$, whereas the infiltrating cells were predominantly T cells in $C4d^-$. Glomerular and PTC T cells were cytotoxic phenotype completely.

Keywords

Acute rejection, granzyme B, glomerulitis, macrophage, peritubular capillaritis, T cell

History

Received 7 November 2014
Revised 2 February 2015
Accepted 18 March 2015
Published online 14 April 2015

Introduction

Allograft glomerulitis and peritubular capillaritis (PTCitis) have been correlated with C4d deposition and antibody-mediated rejection and with an increased probability of graft loss.[1]

Inflammatory cells within glomerular and peritubular capillaries (PTCs) are a common morphological finding in allograft rejection. Glomerulitis and PTCitis characteristic with mononuclear cell infiltration of glomerular and PTCs are a well-recognized component of the acute allograft rejection reaction and are observed in a proportion of biopsy specimens showing acute rejection (AR).[2–4] Both T cells and macrophages have been shown in glomerular and PTCs in allografts with transplant glomerulitis and PTCitis.[4–6] Recent studies suggested an association of allograft glomerulitis and PTCitis with acute humoral rejection (AHR),[7] PTC C4d deposition.[6,8,9]

Complement split factor C4d is produced by activation of the classical complement pathway by antigen–antibody complexes and binds covalently to tissue elements at the site of activation. Deposition of C4d along PTC has closely related to circulating donor-specific antibody levels in renal allograft recipients experiencing AR and has been confirmed as a marker for AHR.[10–13] 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] Magil et al.[4,6] discovered that glomerular and PTC macrophages have been shown to be the predominant cell type in transplant glomerulitis and PTCitis in biopsy specimens showing AR with PTC C4d deposition, whereas in C4d negative biopsies which is mainly composed of T cells. Previous studies have confirmed that Granzyme B, a protein released by cytotoxic T lymphocytes, is associated with AR.[15–19] But did not note any significant differences about the cell types between diffuse C4d positive with focal C4d positive transplant glomerulitis and PTCitis. Similarly, also did not find whether there are cytotoxic T cells or regulatory T cells or both.

Objectives

The aim of our study was therefore to quantify and immunohistochemically characterize the glomeruli and PTC cells in different types of rejection: first, to determine whether

Address correspondence to Dr. Qiang He, Department of Nephrology, Zhejiang Provincial People's Hospital, Hangzhou 310014, P.R. China. Tel/Fax: 86-0571-85893689; E-mail: qianghe@zju.edu.cn

accumulation of mononuclear cells in glomeruli and PTC is truly distinctive in different rejection; second, whether in glomeruli and PTC immune cell population, focal C4d-positive rejection is different from that in cases with diffuse C4d-positive rejection and third, whether in glomeruli and PTC, T-cell phenotype is in different rejection and different extent of C4d-positive rejection.

Materials and methods

Patients

Only patients who underwent biopsy within 3–30 days of transplantation between 1 June 2006 and 30 June 2012, and whose allograft biopsy specimens showed both AR according to the Banff '97 criteria[20] and transplant glomerulitis and PTCitis and who had paraffin tissue for immunohistochemistry for C4d, CD3, CD68, granzyme B and Foxp3 were included in this study. Fifty-one biopsy specimens from 51 patients (29 males, 22 females) met selection criteria. It was done after the approval of the Ethic Committee at the Zhe Jiang Provincial People's Hospital and the consent of patients.

Histopathology

All biopsy specimens were formalin fixed (10%, PH 7.2) and paraffin embedded according to the standard procedures. For histopathologic analysis, 2 μm sections were stained with H&E, PAS, Trichrome-stain and Jones's Methenamin Silver.[21] Allograft rejection was assessed and graded according to the Banff classification of renal allograft rejection.[20]

Immunohistochemical examination

Immunohistochemistry was performed on formalin fixed tissue embedded in paraffin. After dewaxing in xylol, endogenous peroxidase activity was blocked with 0.03% H_2O_2. Antigen retrieval was performed using 0.1 M citrate buffer pH 6.0 under pressure, and non-specific reactivity was blocked with normal horse serum. The primary antibodies were rabbit polyclonal antibodies specific for complement split factor C4d (Purchased from Biomedical Corporation, Vienna, Austria), mouse monoclonal antibodies specific for CD3 (a marker for T cells; Clone: 5B10; Dako, Glostrup, Denmark), CD68 (a marker for macrophages; Clone: 236A/E7; Dako), granzyme B (a marker for cytotoxic T cells; Clone: 246A; CHEMICON, Temecula, CA) and Foxp3 (a marker for regulatory T cells; Clone: 36B; Abcam, Cambridge, UK) and were used at a dilution of 1:100. The sections were incubated with the primary antibody at 4 °C overnight. Sections were then incubated for 30 min at room temperature in a proprietary polymer-based secondary antibody (Dako Envision Plus), and then stained with diaminobenzidine (Dako) for 6 MiniSlide were washed with phosphate buffered saline between steps. Sections were counterstained in Harris's hematoxylin for 2 min, rinsed in running tap water for 5 min, dehydrated and mounted with Hystomount. Lymph node tissue was used as positive controls for CD68, CD3, granzyme B and Foxp3. Negative control studies were performed by omitting the primary monoclonal antibody in the staining procedure and using an irrelevant mouse monoclonal antibody as the primary antibody. The criteria for C4d-positive are as our previously described.[22]

Scoring of C4d staining (% of biopsy or five high-power fields)[23]

C4d0: Negative: 0%.
C4d1: Minimal C4d stain/detection: $< 10\%$.
C4d2: Focal C4d stain/positive: 10–50%.
C4d3: Diffuse C4d stain/positive: $> 50\%$.

Quantitative analysis

All biopsies were scored according to the Banff '97 criteria[20] to determine the type and grade of the rejection reaction. The number of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells in glomeruli with full cross-sectioned profiles were counted in each biopsy specimen and expressed as the number of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells per glomerulus. The number of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells in PTCitis in at least six consecutive HPF on sections and expressed as the number of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells per PTC. In addition, the glomerular, PTCs monocyte_T cell biopsy specimens were divided into two groups according to whether they showed diffuse or focal PTC staining for C4d ($C4d^+$ group) or not ($C4d^-$ group). Mean numbers (±SD) of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells per glomerulus and PTC were determined for each group. Mean numbers (±SD) of macrophages, T cells, granzyme B positive T cells and Foxp3 positive T cells per glomerulus and PTC were also determined for diffuse or focal PTC staining for C4d, respectively. Counting was performed independently by two observers blinded to the results of C4d staining and mean values of the two readings were used for calculations.

Statistical analysis

Results are expressed as the mean ± SD. Chi-square test, Fisher exact test, one-way ANOVA and Kruskal–Wallis were used for comparisons between groups. All p-values were two-tailed and a p-value < 0.05 was considered significant.

Results

Patient data and histological features

The characteristics of the two groups are presented in Table 1. Fifty-one patients (22 males, 29 females) had 51 biopsy specimens that showed AR and transplant glomerulitis and PTCitis. Twenty biopsy specimens from 12 males and 14 females showed diffuse ($n = 14$) or focal ($n = 12$) PTC C4d staining ($C4d^+$ group), whereas 25 biopsy specimens from 10 males and 15 females were negative for PTC C4d ($C4d^-$ group). In the $C4d^+$ group, at least 10 glomeruli were available for histological examination in 20 biopsy specimens. In the other six $C4d^+$ biopsy specimens, nine glomeruli were present in four specimens and eight glomeruli were noted in the other two specimens. In the $C4d^-$ group, all except five biopsy specimens contained at least 10 glomeruli. The other biopsy specimens had nine glomeruli each. All biopsy

specimens had at least two interlobular arteries. Distribution of grades of rejection for the $C4d^+$ group (1A, $n =$ Banff15; 1B, $n = 2$; and 2A, $n = 9$) was not significantly different from that of the $C4d^-$ group (1A, $n = 14$; 1B, $n = 1$; and 2A, $n = 10$). There was no significant difference between mean times for biopsy post-transplantation for the $C4d^+$ (12.0 ± 8.8) and $C4d^-$ biopsy specimens (9.8 ± 7.6). Also, no statistically significant differences were observed between two groups for age, gender, duration of cold or warm ischemia, HLA mismatches, complement-dependent-cytotoxic (CDC) result, panel reactive antibody (PRA) > 10% pretransplantation, Biopsy time post-transplantation, serum creatinine values on biopsy time and immunosuppression protocol.

Total number of inflammatory cells in glomeruli and PTCs is distinctive for C4d positive and C4d negative biopsies

Quantitative results are listed in Table 2. Biopsy specimens showed varying numbers of macrophages and T cells in glomerulus and PTC (Figures 1 and 2). An estimate of the total numbers of glomerulus and PTC cells was determined by summing up the counts for CD3 and CD68 positive cells within glomeruli and PTCs. Comparing cell counts in $C4d^-$ and $C4d^+$ glomerulitis and PTCitis biopsies, a significant difference of absolute numbers of intracapillary cells could be observed in glomeruli and PTCs (Figure 3). The total number of inflammatory cells in glomeruli and PTCs is significantly higher in $C4d^+$ biopsies than in $C4d^-$ biopsies ($p < 0.0001$; $p < 0.0001$).

Table 1. Demographic date and clinical characteristics of patients.

Group	$C4d^+$ ($n = 26$)	$C4d^-$ ($n = 25$)
Age (year ± SD)	38.9 ± 10.4	42.7 ± 8.7
Gender (F/M)	(14/12)	(15/10)
Primary renal disease		
Chronic glomerulonephritis	21 (80.7%)	22 (88.0%)
Hypertension nephropathy	0	0
Gout nephropathy	1(3.8%)	1 (4.0%)
SLE	4 (15.4%)	2 (8.0%)
Cold ischemia time (h)	7.8 ± 3.3	8.1 ± 2.3
Warm ischemia time (min)	6.3 ± 1.4	6.0 ± 1.6
HLA mismatches	3.7 + 1.4	3.9 + 0.7
CDC (%)	3.1 + 1.1	3.2 + 1.5
PRA > 10% pretransplantation	4	1
Time post-transplantation (day)	12.0 + 8.8	9.8 + 7.6
Creatinine concentration on biopsy time (μmol/L)	465 + 165	382 + 218
Immunosuppression protocol		
Pred + CsA + Aza	0	0
Pred + CsA + MMF	7	9
Pred + Tac + MMF	17	15
Pred + Rapa + MMF	2	1
Banff 05 classification		
IA	15	14
IB	2	1
IIA	9	10

Notes: All $p > 0.05$, $C4d^+$ versus $C4d^-$; CDC, complement-dependent-cytotoxic; PRA, panel reactive antibody.

Endocapillary macrophages predominate in C4d positive biopsies

Comparing the numbers of CD3-positive and CD68-positive cells within glomeruli and PTCs (Table 2), we found that the number of glomerular and PTC macrophages was higher in cases of $C4d^+$ (13.73 ± 7.03; 4.36 ± 1.85) than in $C4d^-$ group (2.57 ± 1.22; 2.26 ± 1.64) ($p < 0.0001$; $p = 0.001$). However, the number of glomerular and PTC T cells was less in cases of $C4d^+$ (4.05 ± 2.60; 1.29 ± 0.52) than in $C4d^-$ group (5.60 ± 2.81; 2.01 ± 1.02) ($p = 0.023$; 0.031). It is interesting that not only glomerular T cells but also PTC T cells are all granzyme B positive T cells but no one Foxp3 positive T cells (Figures 4 and 5). The number of glomerular and PTC granzyme B positive T cells was less in cases of $C4d^+$ (3.37 ± 2.34; 1.01 ± 0.58) than in $C4d^-$ group (4.27 ± 2.41; 1.98 ± 0.96). The differences, however, did not achieve statistical significance ($p = 0.141$; $p = 0.231$).

Endocapillary macrophages are higher in diffuse C4d positive group than that of focal C4d positive group

The $C4d^+$ biopsies can be divided into two subgroups based on whether there was a diffuse strong or focal moderately strong or strong PTC staining for C4d. Twenty-three biopsies from 11 patients biopsied had a diffuse PTC C4d reaction, 14 biopsies showed focal PTC C4d staining. Comparing cell counts in diffuse $C4d^+$ and focal $C4d^+$ groups, a significant difference of absolute numbers of intracapillary cells could be observed in glomeruli and PTC (Table 3) (23.25 ± 4.71 vs. 14.49 ± 6.86, $p = 0.001$; 7.53 ± 2.38 vs. 4.32 ± 1.89, $p < 0.0001$). The extent of glomerular and PTC infiltration by macrophages as expressed by the mean number of macrophages per glomerulus and PTC in the diffuse $C4d^+$ group (19.62 ± 4.97; 5.89 ± 2.03) was significantly greater than that of the focal $C4d^+$ group (9.11 ± 4.48; 2.67 ± 1.73)

Table 2. Quantitative analysis of biopsy findings for $C4d^+$ and $C4d^-$ groups.

Variable	$C4d^+$	$C4d^-$	p-Value
Mean no. of macrophages+T cells/glomerulus	17.79 ± 7.70	8.17 ± 3.80	<0.0001
Mean no. of macrophages/glomerulus	13.73 ± 7.03	2.57 ± 1.22	<0.0001
Mean no. of T cells/glomerulus	4.05 ± 2.60	5.60 ± 2.81	0.023
Mean no. of macrophages+T cells/PTC	5.64 ± 2.06	3.68 ± 1.52	<0.0001
Mean no. of macrophages/PTC	4.36 ± 1.85	2.26 ± 1.64	0.001
Mean no. of T cells/PTC	1.29 ± 0.52	2.01 ± 1.02	0.031
Mean no. of granzyme B-positive T cells/glomerulus	3.37 ± 2.34	4.27 ± 2.41	0.141
Mean no. of granzyme B-positive T cells/PTC	1.01 ± 0.58	1.98 ± 0.96	0.231
Mean no. of Foxp3-positive T cells/glomerulus	0	0	
Mean no. of Foxp3-positive T cells/PTC	0	0	

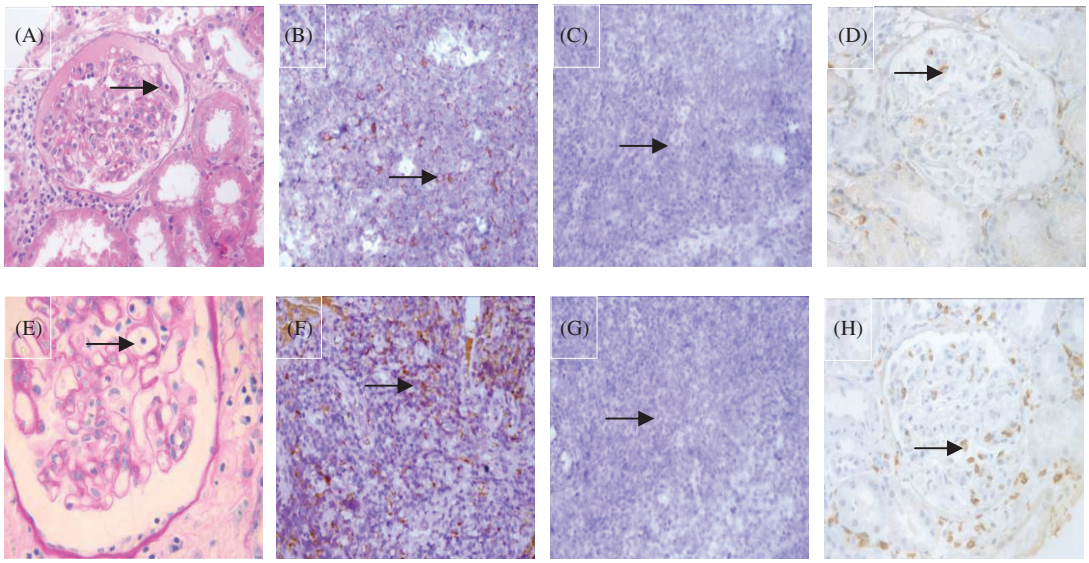

Figure 1. Example of the glomerulitis and immunostaining for two markers (×400, DAB stain × 400): (A) glomerulitis with HE stain (arrow); (B) CD3-positive controls; (C) CD3-negative controls; (D) CD3-positive T lymphocytes in the glomerulus (arrow); (E) glomerulitis with PAS stain (arrow); (F) CD68-positive controls. (G) CD68-negative controls; (H) CD68-positive T lymphocytes in the glomerulus (arrow).

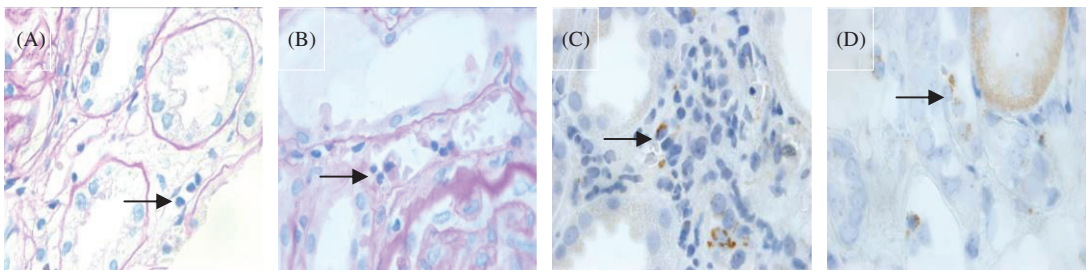

Figure 2. Example of the two PTCitis scores immunostaining for two markers (PAS stain × 1000, DAB stain × 1000). (A) Capillaritis with a PTCitis-score 1, i.e., max 3–4 luminal inflammatory cells in PTC (arrow). (B) Capillaritis with a PTCitis-score 2, i.e., max 5–10 luminal inflammatory cells in PTC (arrow). (C) CD3-positive T lymphocytes in the PTC (arrow). (D) CD68-positive macrophages in the PTC (arrow).

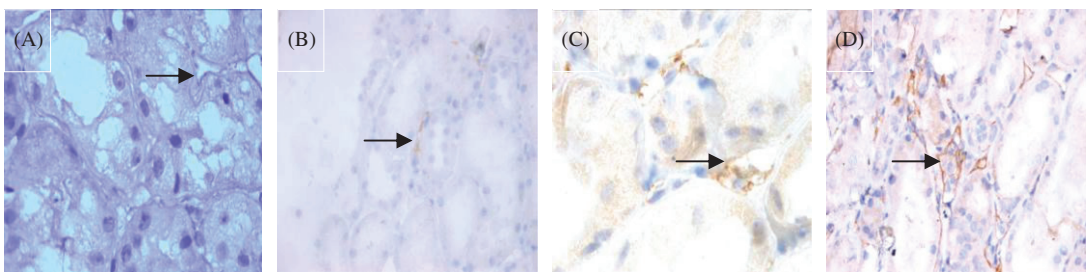

Figure 3. Immunostaining for C4d (DAB stain × 1000). (A) Negative C4d stain in PTC (arrow). (B) Minimal C4d stain/positive in PTC (arrow), i.e., C4d deposition in < 10% PTCs. (C) Focal C4d stain/positive in PTC (arrow), i.e., C4d deposition in 10–50% PTCs. (D) Diffuse C4d stain/positive in PTC (arrow), i.e., C4d deposition in > 50% PTCs.

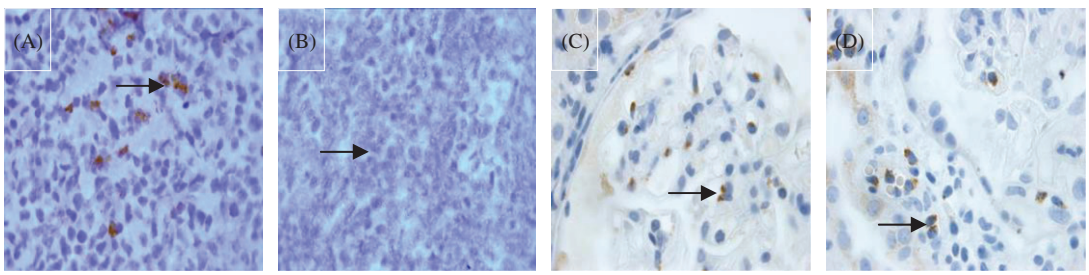

Figure 4. Immunostaining for granzyme-B (DAB stain × 1000). (A) Granzyme B-positive controls (arrow). (B) Granzyme B-negative controls (arrow). (C) Granzyme B-positive T lymphocytes in the glomerulus (arrow). (D) Granzyme B-positive T lymphocytes in the PTC (arrow).

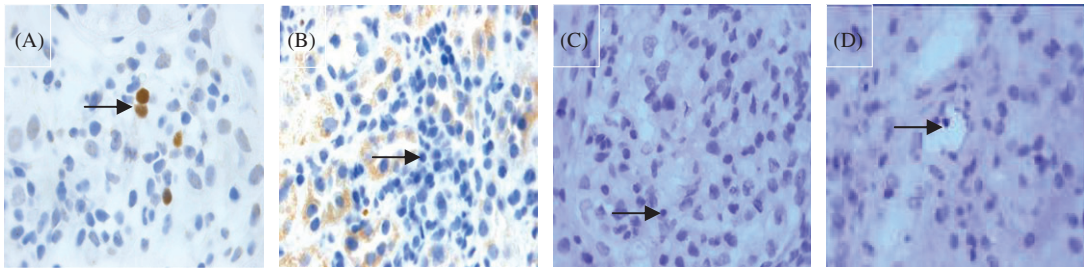

Figure 5. Immunostaining for Foxp3 (DAB stain × 1000). (A) Foxp3-positive controls. (B) Foxp3-negative controls (arrow). (C) Foxp3-negative T lymphocytes in the glomerulus (arrow). (D) Foxp3-negative T lymphocytes in the PTC (arrow).

Table 3. Quantitative analysis of biopsy findings for the diffuse C4d and focal C4d groups.

Variable	Diffuse $C4d^+$	Focal $C4d^-$	*p*-Value
Mean no. of macrophages+T cells/glomerulus	23.25 ± 4.71	14.49 ± 6.86	0.001
Mean no. of macrophages/glomerulus	19.62 ± 4.97	9.11 ± 4.48	<0.0001
Mean no. of T cells/glomerulus	3.64 ± 1.50	4.38 ± 3.24	0.74
Mean no. of macrophages+T cells/PTC	7.53 ± 2.38	4.32 ± 1.89	<0.0001
Mean no. of Macrophages/PTC	5.89 ± 2.03	2.67 ± 1.73	0.001
Mean no. of T cells/PTC	2.97 ± 0.43	2.36 ± 0.98	0.53
Mean no. of granzyme B-positive T cells/glomerulus	2.99 ± 1.36	4.57 ± 2.21	0.215
Mean no. of granzyme B-positive T cells/PTC	1.31 ± 0.45	2.04 ± 0.76	0.182
Mean no. of Foxp3-positive T cells/glomerulus	0	0	
Mean no. of Foxp3-positive T cells/PTC	0	0	

($p < 0.0001$; $p = 0.001$). The number of glomerular and PTC T cells was less in cases of diffuse $C4d^+$ (3.64 ± 1.50; 2.97 ± 0.43) than in focal $C4d^+$ group (4.38 ± 3.24; 2.36 ± 0.98). The differences, however, did not achieve statistical significance ($p = 0.74$; $p = 0.53$). Similarly, glomerular and PTC T cells are all granzyme B positive T cells but no one Foxp3 positive T cells. The number of glomerular and PTC granzyme B positive T cells was less in cases of diffuse $C4d^+$ (2.99 ± 1.36; 1.31 ± 0.45) than in focal $C4d^+$ group (4.57 ± 2.21; 2.04 ± 0.76). The differences, however, did not achieve statistical significance ($p = 0.215$; $p = 0.182$).

Discussion

Renal transplantation is the best form of renal replacement therapy for patients with end stage renal disease resulting in improved survival compared to chronic dialysis treatment. With the continuous advances in immunosuppressive therapy and prophylaxis of infectious complications, there is great improvement of early allograft survival and the long-term survival of renal allografts has improved significantly. AR still remains a problem following kidney transplantation because it is strongly associated with late transplant failure.[24,25]

This is the first study to quantify the relative numbers of glomerular and PTC T cells, macrophages, granzyme B and regulatory T cells in biopsy specimens with transplant glomerulitis and PTCitis and relate the results to PTC C4d deposition level. Early investigations showed both T cells and macrophages in glomeruli and PTCs in specimens, a predominantly macrophages infiltrate in specimens of transplant glomerulitis and PTCitis were associated with PTC C4d deposition, whereas T cells were predominant in specimens of transplant glomerulitis and PTCistis in PTC $C4d^-$ cases.[4,6] As above-mentioned study combined the focal and diffuse $C4d^+$ patients into one group for outcome analysis, no comparison of results with respect to outcome can be made. In present investigation, a predominantly macrophages infiltrate in specimens of transplant glomerulitis and PTCitis were associated with PTC C4d deposition, T cells were predominant in specimens of transplant glomerulitis and PTCitis in PTC $C4d^-$ cases, in agreement with previous reports. Whereas mean number of total cells per glomerulus and PTC and mean number of macrophages per glomerulus and PTC in diffuse $C4d^+$ were higher than in the focal $C4d^+$ group.

A series of studies have elucidated that Granzyme B, proteins released by cytotoxic T lymphocytes, is associated with AR. In contrast, a specialized subset of CD4+CD25+T lymphocytes (Treg cells) seems to suppress autoimmunity and maintain self-tolerance. Noninvasive measurements of urinary Foxp3 mRNA seem to predict the outcome of AR. We studied the immunophenotype of T lymphocytes infiltrating renal biopsy specimens of allografts AR. Our previously study confirmed that the ratio of infiltrating Granzyme B-positive to Foxp3-positive T cells was a sensitive, specific marker distinguishing a ''cytotoxic phenotype'' from a ''regulatory phenotype''. Also, 85.9% of control biopsy specimens presented regulatory phenotypes. However, 96.2% and 92.0% of $C4d^+$ AR and $C4d^-$ AR biopsy specimens presented the cytotoxic phenotype, respectively.[19] In this research, we found glomerular and PTC T cells are all granzyme B positive T cells but no one Foxp3 positive T cells. The number of glomerular and PTC granzyme B positive T cells were less in cases of diffuse than in focal $C4d^+$ group.

Transplant glomerulitis and PTCitis have been associated with AHR[7] and PTC C4d deposition[6,8,9] which has been suggested as a marker of AHR.[10–13] It is tempting to consider transplant glomerulitis and PTCitis as a possible surrogate marker for AHR. However, as shown in this investigation, without knowledge of glomerular and PTC cell types and their relative numbers, this may be misleading. In view of the close correlation of glomerular and PTC macrophages with PTC C4d deposition and the predominance of glomerular T cells in transplant glomerulitis and PTCistis in PTC $C4d^-$ biopsy specimens, one might consider predominantly monocytic transplant glomerulitis and PTCitis as a marker for AHR. Furthermore, it should be noted that the total number of inflammatory cells in glomeruli and PTCs is distinctive between $C4d^+$ and $C4d^-$ groups.

The focus of current immunosuppression is prevention of T-cell activation and infiltration into the transplanted organ.[26] The immunophenotype of the inflammatory cells in AR has long been thought to be predominantly T lymphocytic.[27] However, early studies using cellular markers in AR identified macrophages as a significant component of the mononuclear infiltrate in tubulointerstitial rejection.[12]

While it has long been known that the cellular infiltrate in the tubulointerstitium of AR is predominantly composed of T cells,[28] it is widely recognized that macrophages are also involved in tubulitis, contributing to 38–60% of the inflammatory infiltrate.[29] It has been confirmed by many that the humoral immune has an important role in allograft rejection. Deposition of C4d along PTC has been suggested as a marker for AHR. Several studies also have shown a significant association between PTC C4d deposition and vascular rejection.[29–31] Wenqing et al.[32] discovered that 77.8% acute vacular rejection with C4d deposition along PTC. One previous study noted that the highest numbers of glomerular macrophage were found in cases of vascular rejection.[33] Another study showed that the macrophage is the predominant cell type in renal allograft intimal arteritis from acute vacular rejection biopsies which confirmed that the macrophage has relation with AHR.[34]

The finding of glomerulitis and PTCitis predominantly composed of macrophages provides some insight into potential mechanisms of AR, which has largely been identified as a T-cell derived entity. It may be that only rare T lymphocytes in the subendothelium, or even T cells in the tubulointerstitium, are sufficient to induce glomerulitis PTCitis which is then largely composed of macrophages. Macrophages may contribute to AR and tissue damage by a number of mechanisms, including cell-mediated cytotoxicity,[35] antigen presentation and T-cell activation,[36] production of nitric oxide,[37] and release of inflammatory cytokines.[38] Finally, macrophages may induce myofibroblast proliferation through production of profibrogenic growth factors, leading to interstitial fibrosis and the occurrence of chronic allograft nephropathy.[39]

Macrophages are known to be mediate tissue damage not only in the kidney but also in other organs. There is a growing evidence of their accumulation in graft rejection.[40] In a research using liposomal clodronate to deplete macrophages in which lymphocyte infiltration within the renal allograft was maintained, tissue damage was reduced and renal function was preserved. This shows an important role for the macrophage in graft destruction in AR.[41] This finding confirms that the mechanisms related to injury in AR are not only due to T-cell-mediated cytotoxicity but also may be due, at least in part, to injury derived from macrophages.

The prognostic significance of transplant glomerulitis and PTCitis is uncertain. One previous study discovered that the presence of transplant glomerulitis and PTCistis in biopsy specimens with borderline AR predicted progression to histological AR.[42]

In conclusion, we provide evidence that humoral injury (indicated by endothelial C4d deposits in PTC) is associated with a predominantly macrophage infiltration within glomeruli and PTCs, especially in diffuse C4d deposits in PTC, furthermore, not only glomerular but also PTC T cells are all granzyme B positive T cells but no one Foxp3 positive T cells. In view of the association of transplant glomerulitis and PTCitis with PTC C4d deposition, transplant glomerulitis and PTCitis, especially the macrophage-predominant type, may have a negative impact on graft survival.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This work was funded by National Natural Science Foundation (81170691) and Provincial Foundation Project (WKJ 2013-2-002).

References

1. Lefaucheur C, Nochy D, Hill GS, et al. Determinants of poor graft outcome in patients with antibody-mediated acute rejection. *Am J Transplant*. 2007;7(4):832–841.
2. Marcussen N, Solez K, Spencer E, et al. Early transplant glomerulitis: Glomerular size and ultrastructure. *Transplant Proc.* 1996;28:468–469.
3. Olsen S, Spencer E, Cockfield S, et al. Endocapillary glomerulitis in the renal allograft. *Transplantation*. 1995;59:1421–1425.
4. Fahim T, Bohmig GA, Exner M, et al. The cellular lesion of humoral rejection: Predominant recruitment of monocytes to peritubular and glomerular capillaries. *Am J Transplant.* 2007;7: 385–393.
5. Bishop GA, Hall BM, Duggin GG, et al. Immunopathology of renal allograft rejection analyzed with monoclonal antibodies to mononuclear cell markers. *Kidney Int*. 1986;29:708–717.
6. Magil AB, Tinckam K. Monocytes and peritubular capillary C4d deposition in acute renal allograft rejection. *Kidney Int*. 2003;63: 1888–1893.
7. Trpkov K, Campbell P, Pazderka F, et al. Pathologic features of acute renal allograft rejection associated with donor-specific antibody. *Transplantation.* 1996;61:1586–1592.
8. Nickeleit V, Zeiler M, Gudat F, et al. Detection of the complement degradation product C4d in renal allografts: Diagnostic and therapeutic implications. *J Am Soc Nephrol.* 2002;13:242–251.
9. Sund S, Hovig T, Reisaeter AV, et al. Complement activation in early protocol kidney graft biopsies after living-donor transplantation. *Transplantation*. 2003;75:1204–1213.
10. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: Diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol*. 1999;10:2208–2214.
11. Mauiyeddi SA, Crespo M, Collins AB, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol.* 2002;13: 779–787.
12. Böhmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allografts: A specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol*. 2002;13:1091–1099.

13. Feucht HE. Complement C4d in graft capillaries – The missing link in the recognition of humoral alloreactivity. *Am J Transplant.* 2003; 3:646–652.
14. Racusen LC, Colvin RC, Solez K, et al. Antibodymediated rejection criteria – An addition to the Banff '97 classification of renal allograft rejection. *Am J Transplant*. 2003;3:708–714.
15. Mauiyyedi S, Crespo M, Collins AB, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol*. 2002;13: 779–785.
16. Wevwer PC, Aten J, Rentenaar RJ, et al. Apoptotic tubular cell death during acute renal allograft rejection. *Clin Nephrol*. 1998;49: 28–34.
17. Vasconcellos LM, Schachter AD, Zheng XX, et al. Cytotoxic lymphocyte gene expression in peripheral blood leukocytes correlates with rejecting renal allografts. *Transplantation.* 1998;66: 562–566.
18. Li B, Hartono C, Ding R, et al. Noninvasive diagnosis of renal allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med.* 2001;344:947–954.
19. Jin J, Xu Y, Wang H, et al. Peritubular capillaritis in early renal allograft dysfunction is an indicator of acute rejection. *Transplant Proc.* 2013;45:163–171.
20. Racusen L, Solez K, Colvin R, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int.* 1999;55: 713–723.
21. Grimbert P, Mansour H, Desvaux D, et al. The regulatory/ cytotoxic graft-infiltrating T cells differentiate renal allograft borderline change from acute rejection. *Transplantation*. 2007;83: 341–346.
22. Jianghua C, Wenqing X, Huiping W, et al. C4d as a significant predictor for humoral rejection in renal allografts. *Clin Transplant*. 2005;19:785–791.
23. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8:753–760.
24. Hariharan S, McBride MA, Cherikh WS, et al. Post-transplant renal function in the first year predicts long-term kidney transplant survival. *Kidney Int.* 2002;62:311–316.
25. Meier-Kriesche HU, Schold JD, Kaplan B, et al. Long-term renal allograft survival: Have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant.* 2004;4:1289–1295.
26. Kaufman DB, Shapiro R, Lucey MR, et al. Immunosuppression: Practice and trends. *Am J Transplant.* 2004;4:38–53.
27. Underhill DM, Bassetti M, Rudensky A, et al. Dynamic interactions of macrophages with T cells during antigen presentation. *J Exp Med.* 1999;190:1909–1914.
28. Bishop GA, Hall BM, Duggin GG, et al. Immunopathology of renal allograft rejection analyzed with monoclonal antibodies to mononuclear cell markers. *Kidney Int.* 1986;29:708–717.
29. Herzenberg AM, Gill JS, Djurdjev O, et al. C4d deposition in acute rejection: An independent long-term prognostic factor. *J Am Soc Nephrol.* 2002;13:234–241.
30. Feucht HE, Schneeberger H, Hillebrand G, et al. Capillary deposition of C4d complement fragment and early graft loss. *Kidney Int.* 2003;43:1333–1338.
31. Kluth-Pepper B, Schneeberger H, Lederer SR, et al. Impact of human alloreactivity on the survival of renal allografts. *Transplant Proc.* 1998;30:1772.
32. Jianghua C, Wenqing X, Huiping W, et al. C4d as a significant predictor for humoral rejection in renal allografts. *Clin Transplant.* 2005:19:785–791.
33. Michaels PJ, Espejo ML, Kobashigawa J, et al. Humoral rejection in cardiac transplantation: Risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J Heart Lung Transplant*. 2003;22:58–69.
34. Matheson PJ, Dittmer ID, Beaumont BW, et al. The macrophage is the predominant inflammatory cell in renal allograft intimal arteritis. *Transplantation.* 2005;79:1658–1662.
35. Tesch GH, Schwarting A, Kinoshita K, et al. Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. *J Clin Invest.* 1999;103:73–80.
36. Underhill DM, Bassetti M, Rudensky A, et al. Dynamic interactions of macrophages with T cells during antigen presentation. *J Exp Med.* 1999;190:1909–1914.
37. Langherhr JM, White DA, Hoffman RA, et al. Macrophages produce nitric oxide at allograft sites. *Ann Surg.* 1993;218:159–166.
38. Vandenbrodcke C, Caillat-Zucman S, Legendre C, et al. Differential *in situ* expression of cytokines in renal allograft rejection. *Transplantation.* 1991;51:602–609.
39. Pilmore HL, Yan Y, Eris JM, et al. Time course of upregulation of fibrogenic growth factors and cellular infiltration in a rodent model of chronic renal allograft rejection. *Transpl Immunol*. 2002;10: 245–254.
40. Le Meur Y, Jose MD, Mu W, et al. Macrophage colony-stimulating factor expression and macrophage accumulation in renal allograft rejection. *Transplantation.* 2002;73:1318–1324.
41. Jose MD, Ikezumi Y, van Rooijen N, et al. Macrophages act as effectors of tissue damage in acute renal allograft rejection. *Transplantation.* 2003;76:1015–1022.
42. Meehan SM, Siegel CT, Aronson AJ, et al. The relationship of untreated borderline infiltrates by the Banff criteria to acute rejection in renal allograft biopsies. *J Am Soc Nephrol*. 1999;10: 1806–1814.