Increased influx of myeloid dendritic cells during acute rejection is associated with interstitial fibrosis and tubular atrophy and predicts poor outcome

Kim Zuidwijk[1], Johan W. de Fijter[1], Marko J.K. Mallat[1], Michael Eikmans[2], Marian C. van Groningen[3,5], Natascha N. Goemaere[4], Ingeborg M. Bajema[3] and Cees van Kooten[1]

[1]Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands and [4]Laboratory for Pathology, PATHAN Foundation, Rotterdam, The Netherlands

Dendritic cells are key players in renal allograft rejection and have been identified as an intrinsic part of the kidney. Here we quantified and phenotyped the dendritic cell populations in well-defined biopsies of 102 patients with acute renal allograft rejection in comparison with 78 available pretransplant biopsies. There was a strong increase in BDCA-1$^+$ and DC-SIGN$^+$ myeloid, BDCA-2$^+$ plasmacytoid, and DC-LAMP$^+$ mature dendritic cells in rejection biopsies compared with the corresponding pretransplant tissue. Mature dendritic cells were mostly found in clusters of lymphoid infiltrate and showed a strong correlation with the Banff infiltrate score. The presence of both myeloid and plasmacytoid dendritic cell subsets in the kidney during acute rejection correlated with interstitial fibrosis and tubular atrophy. Importantly, the myeloid dendritic cell density at the time of acute rejection was an independent risk factor for loss of renal function after the first year. Thus, acute renal allograft rejection is characterized by an influx of myeloid and plasmacytoid dendritic cells, strongly associated with local damage in the graft. Hence, the density of myeloid dendritic cells during acute rejection could be an important risk factor for the long-term development of chronic changes and loss of graft function.

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Correspondence: *Cees van Kooten, Department of Nephrology, Leiden University Medical Center, C3-P, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands. E-mail: kooten@lumc.nl*

[5]Current address: Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands.

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Acute renal allograft rejection is associated with an increase of recipient leukocytes infiltrating the interstitium of transplanted kidneys.[1] The severity of acute rejection is scored according to the intensity and distribution of infiltrating cells, whereas their phenotype is not taken into consideration. Recent studies on characterization of lymphocyte subsets in rejection biopsies revealed that determination of the phenotype of infiltrating cells in acute rejection biopsies allows a patient-specific risk estimation and a more tailor-made therapeutic intervention.[2–4]

Although graft-infiltrating lymphocytes are considered as the main effector cells during the rejection process,[5] the immune response is initiated by antigen-presenting cells (APCs).[6,7] Recent studies have indicated the importance of the presence and composition of APCs in graft outcome, showing these cells as the predominant cell type infiltrating the kidney during allograft rejection.[8–11] Human studies have mainly focused on the presence of monocytes and macrophages, indicating the presence of these cells as an independent marker for acute rejection.[12,13] The finding that the influx of monocytes and macrophages during acute rejection is more closely associated with the degree of renal dysfunction than the infiltrating T cells implicates these APCs as critical effector cells during acute rejection.[8]

Dendritic cells (DCs) are professional APCs, which are the main cells responsible for T-cell activation.[14–17] Two major subsets of immature DCs have been described, myeloid DCs (mDCs), defined as CD11c$^+$ and blood dendritic cell antigen 1 positive (BDCA-1$^+$),[18] and plasmacytoid DC (pDCs), expressing CD123 and blood dendritic cell antigen 2 (BDCA-2).[18–21] Once activated, mDCs are primarily involved in the activation of donor-specific T cells, whereas pDCs have been described to be involved in tolerance induction in allotransplantation of solid organs.[22–24] However, interaction of T cells and pDCs in the spleen appears to be sufficient to induce allograft rejection.[25] Mature mDCs are potent stimulators of type 1 helper T cell and cytotoxic T-lymphocyte responses, and are therefore thought to be the predominant cell type mediating acute graft rejection.[20]

The importance of DCs in the induction of alloresponses following kidney transplantation has been indicated by depletion and reconstitution studies.[26] Murine studies showed that the presence of DCs in the kidney could be critical in determining graft outcome.[11] Identification of DCs in the human kidney revealed the presence of low numbers of different immature DC subsets, being BDCA-1$^+$ DC-SIGN$^+$ and BDCA-1$^+$ DC-SIGN$^-$ mDCs and BDCA-2$^+$ pDCs.[27] However, despite the importance ascribed to APCs in determining graft outcome, there is little information on the presence and composition of DCs in the human kidney during renal allograft rejection. In the present study, we show a significant increase in the number of mDCs, pDCs, and mature DCs during acute renal allograft rejection and their relation to clinical and histological parameters.

RESULTS

The identification of DC subsets in kidney rejection biopsies

Kidney biopsies of the 102 patients with a first acute rejection episode were evaluated for the presence of mDCs, pDCs, and mature DCs. For comparison, the available pretransplant biopsies ($n = 78$) of these patients were used. There was no difference in baseline characteristics for the patients with or without a pretransplant biopsy available. The population studied had a representative age and gender distribution for renal transplant recipients, was relatively well matched, and 25% of the patients received a living donor kidney (Table 1). All cases with delayed graft function (DGF; 28%) occurred in recipients of deceased donor kidneys and 48% of the patients with rejection required antibody therapy with antithymocyte globulin because of insufficient response to steroid treatment or a second rejection episode.

The presence of mDCs was evaluated by immunohistochemical staining for BDCA-1 and DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing nonintegrin). In both pretransplant and rejection tissue, BDCA-1$^+$ and DC-SIGN$^+$ cells were localized around glomeruli and in the tubulointerstitium, but were rarely within the glomeruli (Figure 1a–d). In comparison with biopsy samples of pretransplant tissue, mDCs were more abundantly present during acute rejection episodes. As reported previously, more BDCA-1$^+$ cells than DC-SIGN$^+$ cells were found in pretransplant biopsies.[27] In rejection tissue, however, the amount of DC-SIGN$^+$ cells exceeded the number of BDCA-1$^+$ cells.

Upon staining for BDCA-2, a marker specific for pDCs, the tubulointerstitium of pretransplant biopsies was found to contain pDCs, although in a low frequency (Figure 1e). We detected numerous positive cells in rejection biopsies, although a wide variation was found among the different patients evaluated (Figure 1f). The localization of pDCs in both pretransplant and rejection biopsies was comparable to mDCs.

DC-LAMP (DC-lysosomal-associated membrane protein) staining revealed unexpected high numbers of mature DCs in rejection tissue, whereas pretransplant biopsies only showed a

Table 1 | Patient characteristics

Variable	Mean ± s.d.; number (%)
Acceptor	
Age	46.8 ± 12.9
⩽50 years	59 (57.8)
>50 years	43 (42.2)
Sex	
♀	34 (33.3)
♂	68 (66.7)
Donor	
Age	45.7 ± 14.0
Age	
⩽50 years	58 (56.9)
>50 years	44 (43.1)
Sex	
♀	63 (61.8)
♂	39 (38.2)
Donor source	
Living	25 (24.5)
Postmortem	77 (75.5)
Retransplantation	
No	87 (85.3)
Yes	15 (14.7)
DGF (need for post-Tx dialysis)[a]	
No	73 (71.6)
Yes	29 (28.4)
Induction therapy	
No	71 (69.6)
Yes	31 (30.4)
Need for antibody therapy	
No	53 (52.0)
Yes	49 (48.0)
PRA (%)	
0–5%	34 (33.3)
>5%	68 (66.7)
Mismatches	
Class I	
0–2	78 (79.6)
>2	20 (20.4)
Class II	
0	32 (33.0)
1–2	65 (67.0)
Patient survival	
1 year	99/102 (97.0 ± 0.02%)
5 years	89/102 (86.7 ± 0.04%)
Graft survival	
1 year	97/102 (95.1 ± 0.02%)
5 years	92/102 (89.5 ± 0.03%)
Graft function at 1 year	
<50 ml/min	67 (65.7), 35.3 ± 9.21[b]
⩾50 ml/min	35 (34.3), 58.7 ± 6.37

Abbreviations: DGF, delayed graft function; PRA, panel reactive antibodies; Tx, transplant.
[a]All cases of DGF were observed in the group transplanted with kidneys from deceased donors.
[b]A total of seven patients were excluded from this analysis because of graft failure and/or death within the first year.

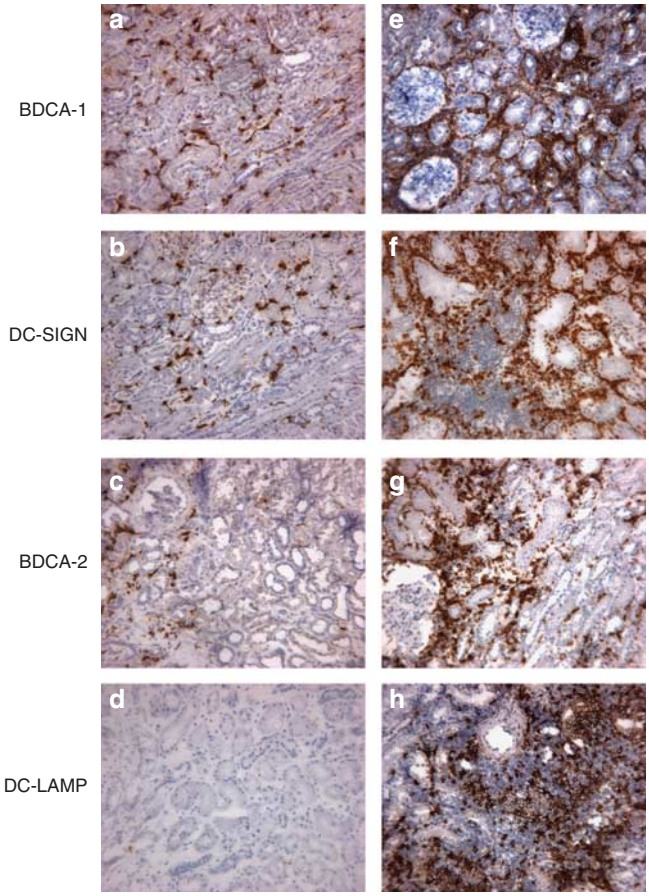

Figure 1 | Myeloid, plasmacytoid, and mature dendritic cells (DCs) are increased in rejection biopsies. Cryosections of (**a**–**d**) pretransplant and (**e**–**h**) rejection tissues were stained immunohistochemically for (**a**, **e**) BDCA-1, (**b**, **f**) DC-SIGN, (**c**, **g**) BDCA-2, and (**d**, **h**) DC-LAMP as described in the Materials and Methods. Original magnification ×100. BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin.

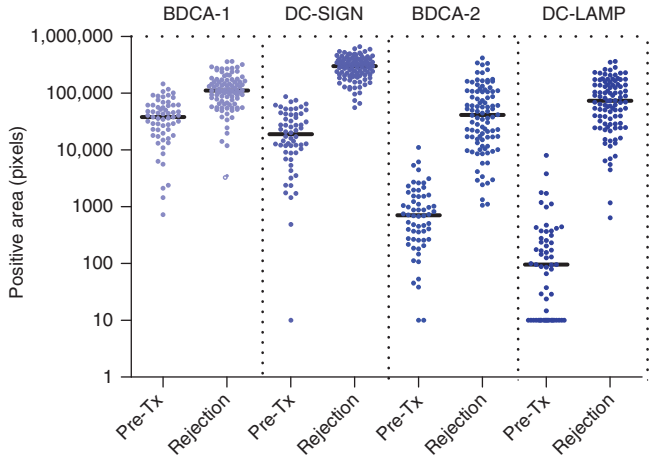

Figure 2 | Quantification of dendritic cell (DC) subsets in pretransplant and rejected kidneys. Cryosections of pretransplant (pre-Tx; $n = 78$) and rejected kidneys ($n = 104$) were stained for BDCA-1, DC-SIGN, BDCA-2, and DC-LAMP as described in the Materials and Methods. Single dots represent the positive areas in each section/biopsy. Horizontal lines represent the median expression. The presence of DC-LAMP-positive cells was <100 pixels/area in 42 biopsies. BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin.

limited number of positive cells (Figure 1g and h). Mature DCs showed a unique distribution pattern, as the majority of these cells were observed within clusters of lymphocytic infiltrate.

Quantification of DCs in kidney biopsies

To quantify the number of DCs in different tissues, digital image analysis was used. We observed that mDCs were abundantly present in pretransplant tissue with a median of 3.8×10^4 BDCA-1$^+$ pixels/area (range: 0.1–14.6×10^4), but especially during allograft rejection with a median of 11.1×10^4 BDCA-1$^+$ pixels/area (range: 0.4×10^4–36.7×10^4; Figure 2). DC-SIGN$^+$ cells were found in pretransplant tissue with a median of 1.9×10^4 DC-SIGN$^+$ pixels/area (range: 0–8.7×10^4) and in rejection tissue with a median of 29.9×10^4 DC-SIGN$^+$ pixels/area (range: 5.6–66.6×10^4).

PDCs cells were about three times less frequently present in rejection tissue compared with mDCs, with a median of 4.1×10^4 BDCA-2$^+$ pixels/area (range: 0.1×10^4–41.8×10^4; Figure 2). In pretransplant tissue, pDCs were only observed in low frequencies with a median of 0.06×10^4 (range: 0–1.1×10^4).

The availability of paired pretransplant and rejection biopsies of 78 patients provided the opportunity to calculate the relative increase during acute rejection. The number of both mDCs and pDCs were significantly increased in rejection tissue ($P < 0.0001$ for both subsets). For mDCs, a 3- and 15-fold increase was found for BDCA-1$^+$ and DC-SIGN$^+$ cells, respectively, in rejection biopsies compared with their corresponding pretransplant tissue. An even stronger increase (median 65-fold) was observed for pDCs (Figure 3).

Quantification of mature DCs in rejection tissue revealed that these cells were present with a median of 7.4×10^4 DC-LAMP$^+$ pixels/area (range: 0.06×10^4–36.6×10^4), whereas almost no positive cells were found in pretransplant tissue with a median of 0.01×10^4 positive pixels/area (range: 0–0.8×10^4; Figure 2). Mature DCs showed a 750-fold increase when rejection tissue was compared with the corresponding pretransplant biopsy (Figure 3).

In rejection tissue, the strongest correlation was found between BDCA-1$^+$ and DC-SIGN$^+$ mDCs ($r = 0.66$, $P < 0.0005$), possibly representing double-positive myeloid cells.[27] A significant correlation was also found between BDCA-1$^+$ and BDCA-2$^+$ cells ($r = 0.44$, $P < 0.0005$), despite the fact that these represent distinct populations. DC-LAMP significantly correlated with all three populations: BDCA-1

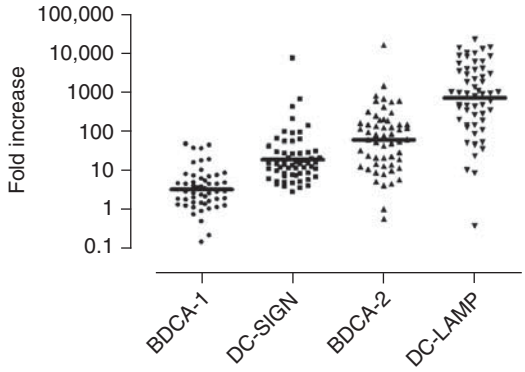

Figure 3 | Increase of dendritic cells (DCs) in pretransplant vs. rejection tissue. The presence of cells positive for BDCA-1, DC-SIGN, BDCA-2, and DC-LAMP was quantified for all coupled rejection and pretransplant biopsies. Single dots represent the fold increase for each rejection–pretransplant pair. Horizontal lines represent the median fold increase. BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin.

($r = 0.48$, $P < 0.0005$), DC-SIGN ($r = 0.43$, $P < 0.0005$), and BDCA-2 ($r = 0.36$, $P < 0.0005$).

Clinical associations of different DC subsets and transplant characteristics

We determined whether recipient and donor characteristics (Table 1) were related to the numbers of DCs at the time of acute rejection (Table 2). The density of mature DCs negatively correlated with the age of the recipient ($P = 0.059$), possibly reflecting immunosenescence associated with increasing age.[28,29] Immunological risk factors including histoincompatibility (HLA mismatch) or induction therapy did not correlate with the number of DCs during acute rejection, whereas sensitization (panel reactive antibodies) was associated with increased numbers of pDCs ($P = 0.045$). We found an association between the presence of DC-SIGN$^+$ mDCs and pDCs and the occurrence of DGF ($P = 0.052$ and 0.029, respectively). A similar association was found when the subgroup of patients with a living donor was excluded from analysis. The number of DCs in the kidney during rejection was not related to the subsequent need for antibody therapy. In multivariable analysis, both panel reactive antibodies and mismatch of class II remained independently associated with pDC numbers (Table 3).

Correlation of different DC subsets and pathological parameters

We next investigated correlations between the presence of DC subsets and histological scores (Table 4). As the DC subsets analyzed were mainly present in the tubulointerstitial compartment of the kidney, and rarely observed within glomeruli, we focused on the tubulointerstitial scores of the Banff classification.

In the univariate analysis, the number of pDCs and both BDCA-1$^+$ and DC-SIGN$^+$ mDC subsets was strongly associated with the score for interstitial fibrosis ($P = 0.004$, 0.017, and < 0.0005, respectively; Table 2). In addition, pDCs and DC-SIGN$^+$ mDCs showed a significant association with the presence of tubular atrophy ($P = 0.015$ and 0.010, respectively; Table 2). Multivariable analysis confirmed the significant correlation between the presence of BDCA-1$^+$ and DC-SIGN$^+$ mDCs and interstitial fibrosis ($P = 0.016$ and < 0.0005, respectively). In both uni- and multi-variable analyses, a very strong correlation was found between the total inflammation score and all DC subsets investigated.

The presence of DC-SIGN$^+$ mDCs was also related to the infiltrate score ($P < 0.0005$). Importantly, the presence of mature DCs was not associated with chronic changes, but correlated strongly with the pathological score for interstitial infiltrate in univariate analysis ($P = 0.006$; Table 2), which is in line with the observation that mature DCs were mainly present in clusters of infiltrating cells (Figure 1h).

With regard to indications of acute humoral rejection, the presence of C4d was associated with reduced numbers of pDCs, whereas the capillaritis score, but not the type of peritubular capillary infiltrate, was associated with increased numbers of myeloid and mature DCs (Table 2).

The presence of DCs at the time of rejection is predictive of long-term graft function

Finally, we investigated the predictive value of the presence of DC subsets and evolution of renal function. Inferior outcome at 1 year after transplantation is defined as allograft loss or an estimated glomerular filtration rate of < 50 ml/min. Renal function at 1 year was significantly affected by classical risk factors, including donor age, occurrence of DGF, and rejection requiring antibody therapy (Table 5). At the tissue level, the presence of tubular atrophy was predictive of inferior function at 1 year. No significant effect on renal function at 1 year was observed for the presence of DC subsets during rejection.

We next investigated the impact on the progression of renal function beyond the first year, defined as a doubling of serum creatinine relative to the 1-year level, or return to dialysis. As expected, serum creatinine values at 1 year posttransplantation were predictive of reaching the defined end point (hazard ratio (HR) = 1.007/unit serum creatinine, $P = 0.001$). Of the other clinical and pathological parameters, despite the relatively small number of patients, a clear tendency was found for donor age, interstitial fibrosis, and tubular atrophy.

Importantly, progressive loss of renal function was significantly associated with the density of BDCA-1$^+$ (HR = 1.064/10^4 pixels, $P = 0.027$) and DC-SIGN$^+$ DCs (HR = 1.039/10^4 pixels, $P = 0.034$). The presence of pDCs was borderline significant (HR = 1.044/10^4 pixels, $P = 0.056$). No correlation was found with the presence of mature DCs during acute rejection (Table 6). In addition, in the multivariable analysis, the presence of DC-SIGN$^+$ DCs was independently associated with progressive loss of renal function (HR = 1.052/10^4 pixels, $P = 0.010$; Table 6).

Table 2 | Univariate associations between DC quantification and demographic/pathological characteristics

	BDCA-1			DC-SIGN			BDCA-2			DC-LAMP		
Variable	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value
Acceptor age			0.305			0.709			0.203			0.059
⩽50 years	13.6	11.8–16.1		30.4	26.9–33.8		8.1	6.2–11.5		11.1	9.3–13.7	
>50 years	11.8	9.8–15.0		31.3	27.6–35.1		6.0	4.5–9.3		7.8	6.0–11.0	
Acceptor gender			0.692			0.566			0.625			0.800
♀	13.4	10.8–17.6		31.8	27.0–36.7		7.8	5.3–14.6		9.4	7.4–12.9	
♂	12.6	11.1–14.8		30.3	27.3–33.2		7.0	5.5–9.3		9.8	8.2–12.3	
Donor age			0.176			0.506			0.057			0.292
⩽50 years	11.8	10.3–13.9		30.0	26.5–33.5		5.8	4.4–8.7		10.5	8.8–13.0	
>50 years	14.2	11.8–17.8		31.8	28.1–35.4		9.0	6.8–13.0		8.6	6.7–12.0	
Donor gender			0.359			0.358			0.312			0.465
♀	13.5	11.6–16.1		31.7	28.4–35.1		7.9	6.0–11.4		10.2	8.5–12.6	
♂	11.8	10.0–14.5		29.3	25.5–33.1		6.2	4.8–8.8		8.8	6.8–12.6	
Donor type			0.656			0.694			0.667			0.073
Living	13.5	10.5–18.9		29.9	23.2–36.6		6.6	4.2–15.8		12.5	9.7–17.7	
PM	12.6	11.2–14.6		31.1	28.5–33.6		7.4	6.0–9.8		8.7	7.3–10.7	
Retransplant			0.304			0.786			0.257			0.288
No	12.5	11.0–14.4		30.9	28.3–33.5		6.8	5.5–8.9		9.3	7.9–11.1	
Yes	15.2	11.2–23.6		29.9	21.7–38.2		9.7	5.8–29.1		12.1	8.2–23.4	
Induction therapy			0.124			0.820			0.414			0.128
No	12.0	10.5–13.8		30.6	27.7–33.5		6.8	5.3–9.2		8.8	7.3–10.8	
Yes	14.9	12.0–19.7		31.2	26.1–36.4		8.2	5.8–14.5		11.8	9.2–16.5	
PRA			0.355			0.103			**0.045**			0.541
0–5%	11.7	9.9–14.5		27.8	24.1–31.6		5.1	3.8–7.6		8.9	7.2–11.5	
>5%	13.4	11.6–16.0		32.3	29.0–35.5		8.3	6.5–11.5		10.0	8.3–12.7	
Mismatches Class I			0.818			0.475			0.464			0.831
0–2	12.9	11.3–15.1		31.2	28.4–34.1		6.9	5.4–9.4		9.7	8.2–11.9	
⩾3	13.4	10.4–18.8		28.9	23.0–34.8		8.4	5.7–16.2		9.2	6.5–15.7	
Mismatches Class II			0.128			0.193			0.061			0.362
0	11.2	9.5–13.9		28.4	24.8–32.1		5.2	3.9–7.7		8.5	6.6–12.0	
>0	14.0	12.0–16.6		32.1	28.7–35.5		8.3	6.4–11.6		10.2	8.5–12.9	
DGF			0.529			0.052			**0.029**			0.554
No	12.6	11.0–14.7		29.2	26.2–32.2		6.1	4.7–8.5		10.0	8.4–12.3	
Yes	13.9	11.1–18.4		34.7	30.4–39.0		10.3	7.6–16.3		8.8	6.7–13.1	
Need for Ab therapy			0.086			0.135			0.076			0.605
No	11.4	9.7–14.0		28.9	25.6–32.3		5.8	4.4–8.6		9.2	7.6–11.8	
Yes	14.4	12.3–17.3		32.8	29.0–36.5		8.7	6.6–12.6		10.1	8.2–13.3	
C4d			0.860			0.136			**0.027**			0.303
Neg.	12.8	11.2–14.9		29.8	27.1–32.5		7.8	6.3–10.3		9.3	7.9–11.4	
Pos.	13.3	10.7–17.7		35.6	27.1–44.2		3.3	2.0–8.4		12.3	8.8–20.3	
Interstitial Fibrosis[b]												
No	11.2	9.5–13.5	**0.017**	26.3	22.9–29.7	**<0.0005**	5.2	4.0–7.5	**0.004**	8.9	7.3–11.5	0.341
Yes	15.4	13.2–18.5		36.7	33.8–39.6		9.9	7.5–14.4		10.6	8.6–13.9	
Infiltrate[b]												
No	11.0	9.0–14.0	0.053	25.4	22.6–28.3	**<0.0005**	5.9	4.2–10.1	0.157	7.0	5.5–9.9	**0.006**
Yes	14.3	12.5–16.8		35.0	31.4–38.6		8.2	6.4–11.3		11.7	9.9–14.4	
Total inflammation score (ti-score)[b]			**0.013**			**<0.0005**			**<0.0005**			**<0.0005**
t0–t1	10.9	9.3–13.1		26.4	23.6–29.3		4.7	3.5–7.1		6.7	5.4–8.8	
t2–t3	15.3	13.0–18.7		35.4	31.4–39.5		10.5	8.0–15.1		13.2	11.0–16.6	
Tubuli Atrophy[b]												
No	12.3	10.5–14.9	0.462	27.7	24.3–31.1	**0.010**	5.4	3.9–8.9	**0.015**	10.1	8.2–13.0	0.633
Yes	13.6	11.5–16.6		34.3	30.7–37.8		9.3	7.3–12.7		9.2	7.5–12.0	
Tubulitis[b]												
No	11.8	9.9–14.7	0.293	28.0	24.4–31.7	0.062	6.5	4.9–9.6	0.426	9.1	7.2–12.4	0.567
Yes	13.7	11.7–16.3		32.9	29.5–36.2		7.8	5.9–11.3		10.1	8.4–12.7	
Vascular Intimal arteritis[b]			0.154			0.426			0.416			0.899
No	11.4	9.9–13.4		29.7	26.4–33.1		6.2	4.7–9.3		9.5	7.8–12.1	
Yes	14.0	11.5–17.7		31.9	27.7–36.2		7.5	5.5–12.1		9.3	7.1–13.4	

Table 2 | Continued

Variable	BDCA-1			DC-SIGN			BDCA-2			DC-LAMP		
	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value	Mean[a]	95% CI[a]	*P*-value
Capillaritis in PTCs[c]												
Infiltration type			0.084			0.077			0.164			0.260
No infiltration	10.0	7.7–14.2		25.2	20.3–30.1		4.7	3.2–8.9		7.1	5.3–11.0	
Lymphocytes	12.5	9.9–16.8		32.1	27.8–36.5		7.9	5.4–14.8		9.8	7.4–14.5	
Granuloc./mixed	14.6	12.6–17.4		31.6	27.8–35.3		8.0	6.0–11.9		10.4	8.5–13.6	
Infiltration score[d]			**0.027**			**0.008**			0.126			**0.032**
PTC 0	10.0	7.7–14.2		25.2	20.3–30.1		4.7	3.2–8.9		7.1	5.3–11.0	
PTC 1	12.4	10.6–15.0		29.7	26.4–33.0		8.5	6.3–12.7		8.6	6.8–11.5	
PTC $\geqslant 2$	16.6	13.7–21.1		36.3	31.3–41.3		6.9	4.8–12.2		13.7	10.9–18.3	

Abbreviations: BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; CI, confidence interval; DC, dendritic cell; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin; DGF, delayed graft function; PM, postmortem; PRA, panel reactive antibodies; PTC, peritubular capillaries.
[a]Values are given as 10^4 pixels/area.
[b]Yes/no based on dichotomy in Table 4.
[c]Only overall *P*-values are shown.
[d]No differentiation was made for focal and diffuse infiltration in the analysis.
Significant *P*-values are given in bold.

Table 3 | Associations between DC quantifications and demographic/pathological characteristics after multivariable analysis[a]

Variable	BDCA-1			DC-SIGN			BDCA-2			DC-LAMP		
	Mean[b]	95% CI[b]	*P*-value[c]	Mean[b]	95% CI[b]	*P*-value[c]	Mean[b]	95% CI[b]	*P*-value[c]	Mean[b]	95% CI[b]	*P*-value[c]
PRA									**0.022**			
0–5%							4.8	3.3–8.4				
>5%							8.3	5.9–15.0				
Mismatches									**0.035**			
Class II												
0							4.5	3.2–8.0				
>0							8.4	5.9–15.1				
C4d						**0.019**			**0.009**			
Neg.				29.6	25.3–33.8		7.8	5.6–13.3				
Pos.				33.5	26.3–40.8		2.9	1.8–9.8				
Interstitial												
Fibrosis[d]												
No	11.1	9.2–13.9	**0.016**	26.4	21.3–31.5	**<0.0005**						
Yes	15.2	12.6–19.2		35.5	31.5–39.5							
Infiltrate[d]												
No				25.3	21.0–29.6	**0.044**						
Yes				34.1	29.1–39.0							
Total inflammation score (ti-score)[d]			**0.010**			**0.002**			**0.001**			**<0.0005**
t0–t1	10.9	9.2–13.6		26.4	21.8–30.9		4.7	3.3–8.4		6.7	5.4–8.8	
t2–t3	15.4	12.7–19.6		35.3	30.6–40.1		10.8	7.7–19.4		13.2	11.0–16.6	
Tubuli												
Atrophy[d]												
No												
Yes												
Tubulitis[d]												
No												
Yes												

Abbreviations: BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; CI, confidence interval; DC, dendritic cell; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin; PRA, panel reactive antibodies.
[a]Only significant results are shown.
[b]Values are given as 10^4 pixels/area.
[c]Only overall *P*-values are shown.
[d]Yes/no based on dichotomy in Table 4.
Significant *P*-values are given in bold.

Interestingly, the presence of tubulitis at the time of acute rejection appeared to be protective for loss of renal function (HR = 0.387, $P = 0.061$).

In relation to patient survival, only the age of the recipients, as expected, was found to be significant in the multivariable analysis (HR = 3.078, $P = 0.004$) and no

Table 4 | Pathological characteristics according to Banff 07 criteria

Variable	Number (%)
Severity of rejection	
Banff grade I	64 (62.7)
Banff grade II	38 (37.3)
Glomeruli	
% Sclerotic	
0%	65 (65.0)
>0%	35 (35.0)
Chronic glom. change (cg-score)	
No	95 (93.1)
Yes	7 (6.9)
Glomerulitis (g-score)	
No	66 (64.7)
Yes	36 (35.3)
Interstitial space	
Fibrosis (ci-score)	
No	58 (56.9)
Yes	44 (43.1)
Infiltrate (i-score)[a]	
0–1	45 (44.1)
>1	57 (55.9)
Total inflammation (ti-score)[a]	
0–t1	58 (59.2)
t2–t3	40 (40.8)
Tubuli	
Atrophy (ct-score)	
No	54 (52.9)
Yes	48 (47.1)
Tubulitis (t-score)[a]	
0–1	44 (43.1)
>1	58 (56.9)
Vascular pathology	
Arteriolar hyalinosis (ah-score)	
No	68 (66.7)
Yes	34 (33.3)
Intimal arteritis (v-score)	
No	54 (58.7)
Yes	38 (41.3)
Vascular intimal sclerosis (cv-score)	
No	48 (51.1)
Yes	46 (48.9)
Capillaritis in PTCs	
Infiltration type	
No infiltration	25 (25.5)
Lymphocytes	29 (29.6)
Granulocytes/mixed	44 (44.9)
Infiltration score in PTCs	
PTC 0	25 (25.5)
PTC 1 (focal/diffuse)	50 (51.0)
PTC ⩾2 (focal/diffuse)	23 (23.5)
Mesangium	
Matrix increase (mm-score)	
No	74 (73.3)
Yes	27 (26.7)
Acute humoral rejection	
C4d neg./foc.	84 (86.6)
C4d glob. and pos.	13 (13.4)

Abbreviation: PTC, peritubular capillary.
[a]Scores were dichotomized in 0–1 versus 2–3.

correlations were found with the presence of DCs (data not shown).

DISCUSSION

The composition of infiltrating leukocytes at the time of allograft rejection is considered a key factor for the quality and quantity of the alloimmune response, and has been proposed as a potential prognostic factor. Within the population of infiltrating leukocytes, professional APCs including macrophages, B cells, and DCs have been identified as the cells regulating T-cell activation.[5,7,10,30] In the present study, we investigated the presence of different DC subsets in a large cohort of biopsies obtained during a first episode of acute renal allograft rejection. We show that acute renal allograft rejection is characterized by a marked increase of different DC subsets including the presence of mature DCs. In particular, the number of mDCs was associated with the presence of early chronic changes and was predictive of the loss of graft function in the long term.

Although DCs have been recognized as an intrinsic part of the kidney, and are transplanted as passenger leukocytes, more detailed information has become available only recently. Striking information came from CXCR3-GFP mice showing that interstitial DCs form a dense network within the tubulointerstitial compartment of the kidney, a distribution that was confirmed by immunohistochemistry.[31,32] Using pretransplant biopsies of living donor kidneys, we demonstrated a similar network of DC subsets in human kidneys under noninflammatory conditions.[27] Various experimental models have shown that numbers of mDCs increased under inflammatory conditions.[31–33] More recently, it was shown that based on differential expression of CD11c and CD11b, different functional DC subsets can be recognized.[34] In addition, in the present cohort of acute rejection biopsies, different subsets of human mDCs were recognized. The number of BDCA-1$^+$ mDCs correlated strongly with the presence of DC-SIGN$^+$ cells, confirming coexpression of these markers on a subset of mDCs, as demonstrated previously in healthy kidneys.[27] However, in contrast to what was found in pretransplant biopsies, the number of DC-SIGN$^+$ mDCs strongly exceeded the number of BDCA-1$^+$ DCs in rejection tissue. Although DC-SIGN was originally described as a specific DC marker, its expression has also been reported on monocytes early in their differentiation toward DCs.[35–37] A similar population of DC-SIGN+/CD68+ cells was demonstrated in patients with glomerulonephritis.[38] In addition, a population of CD14+/DC-SIGN+ dendritic cells was also recently described.[39] Altogether, these results suggest that the kidney is infiltrated by monocytes during acute rejection, which can function as mDC precursors and can locally develop to mDCs.[40]

In a pilot study, we have looked at the presence of DCs in protocol biopsies taken 6 or 12 months after transplantation in patients who did not experience a rejection episode. We observed that the quantification of DCs was comparable to the analysis seen in pre-transplant biopsies. Unfortunately, we have no protocol biopsies available from the current large

Table 5 | Relationship between DC quantification, demography, and pathology with graft function at 1 year posttransplantation

	eGFR at 1 year univariate			eGFR at 1 year multivariable		
	OR[a]	95% CI	*P*-value	OR[a]	95% CI	*P*-value
Recipient						
Age >50 years	0.802	0.351–1.829	0.599			
Gender ♂	0.582	0.235–1.438	0.241			
Donor						
Age >50 years	**3.160**	**1.289–7.747**	**0.012**	**2.925**	**1.098–7.796**	**0.032**
Gender ♂	1.294	0.552–3.032	0.553			
Transplantation type	0.681	0.253–1.829	0.446			
Retransplantation	1.522	0.447–5.185	0.502			
Induction therapy	1.141	0.466–2.798	0.773			
PRA	0.877	0.366–2.101	0.768			
Mismatch						
Class I	1.588	0.521–4.841	0.416			
Class II	0.478	0.180–1.272	0.139			
Delayed graft function	**3.349**	**1.148–9.767**	**0.027**			
Need for antibody therapy	**3.482**	**1.445–8.391**	**0.005**	**3.789**	**1.444–9.942**	**0.007**
Interstitial space						
Fibrosis	1.457	0.631–3.366	0.378			
Infiltrate	2.240	0.974–5.151	0.058			
Total inflammation score (ti-score)[b]	1.054	0.454–2.446	0.902			
Tubuli						
Atrophy	**4.024**	**1.636–9.898**	**0.002**	**4.289**	**1.614–11.397**	**0.003**
Tubulitis	1.173	0.515–2.672	0.704			
Vascular pathology						
Intimal arteritis	0.832	0.353–1.962	0.675			
Capillaritis in PTCs						
Infiltration type[c]						
Lymphocytes	0.667	0.218–2.041	0.478			
Granulocytes/mixed	0.910	0.320–2.590	0.859			
Infiltration score in PTCs[d]						
PTC 1 (focal/diffuse)	0.913	0.328–2.543	0.863			
PTC ⩾2 (focal/diffuse)	0.612	0.189–1.985	0.413			
C4d pos.	0.553	0.169–1.803	0.326			
DCs[e]						
BDCA-1	1.034	0.977–1.090	0.242			
BDCA-2	1.025	0.970–1.081	0.369			
DC-SIGN	1.004	0.973–1.036	0.794			
DC-LAMP	0.990	0.939–1.040	0.687			

Abbreviations: BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; CI, confidence interval; DC, dendritic cell; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin; eGFR, estimated glomerular filtration rate; OR, odds ratio; PRA, panel reactive antibodies; PTC, peritubular capillaries.
[a]Inferior outcome at 1 year after transplantation is defined as allograft loss or an eGFR of <50 ml/min.
[b]Score t2–t3 vs. t0–t1.
[c]Reference category is: No infiltration.
[d]Reference category is: PTC 0.
[e]ORs and 95% confidence intervals are calculated per 10^4 pixels.
Significant *P*-values are given in bold.

retrospective analysis. Therefore, a new study directly comparing pre, protocol, and rejection biopsies from the same study would be very valuable. However, we feel confident that the observed increase in DC numbers is not the mere result of the transplantation procedure or the immunosuppressive therapy.

Apart from the presence of mDCs, we also confirmed the previously observed presence of pDCs in human kidney biopsies. Quantitative analysis revealed a significant increase in the presence of pDCs in rejection tissue compared with the corresponding pretransplant tissue. pDCs are often mentioned in the context of immune regulation (DC2/DC1 ratio),[41] and have been used for the induction of experimental allograft tolerance.[25] However, pDCs have also been described to be the most important APCs in mediating acute graft versus host disease,[42] as well as in acute rejection of vascularized grafts.[25] In the present study, the presence of pDCs was associated with chronic injury. It will be important to further delineate the functional role of pDCs in the kidney, including the local release of interferon-α, the prototypic product of activated pDCs.[43]

Table 6 | Relationship between DC quantification, demography, and pathology with graft function after the first year posttransplantation

	Graft outcome after year 1 univariate[a]			Graft outcome after year 1 multivariable[a]		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Recipient						
Age > 50 years	0.541	0.178–1.647	0.279			
Gender ♂	0.878	0.339–2.271	0.788			
Donor						
Age > 50 years	2.395	0.927–6.190	0.071			
Gender ♂	1.063	0.410–2.753	0.900			
Transplantation type	3.151	0.724–13.712	0.126			
Retransplantation	2.150	0.692–6.680	0.186			
Induction therapy	2.103	0.683–6.474	0.195			
PRA	1.815	0.594–5.545	0.296			
Mismatch						
Class I	1.302	0.460–3.688	0.619			
Class II	0.741	0.292–1.882	0.529			
Delayed graft function	1.823	0.705–4.719	0.216			
Need for antibody therapy	1.956	0.758–5.049	0.166			
Interstitial space						
Fibrosis	2.205	0.865–5.620	0.098			
Infiltrate	1.285	0.493–3.349	0.607			
Total inflammation score (ti-score)[b]	1.010	0.364–2.803	0.985			
Tubuli						
Atrophy	2.241	0.866–5.800	0.096			
Tubulitis	0.587	0.231–1.490	0.262	0.387	0.143–1.046	0.061
Vascular pathology						
Intimal arteritis	1.454	0.537–3.940	0.462			
Capillaritis in PTCs						
Infiltration type[c]						
Lymphocytes	1.117	0.299–4.171	0.869			
Granulocytes/mixed	1.370	0.391–4.795	0.623			
Infiltration score in PTCs[d]						
PTC 1 (focal/diffuse)	0.957	0.279–3.288	0.944			
PTC ⩾2 (focal/diffuse)	2.290	0.594–8.830	0.229			
C4d pos.	1.691	0.481–5.945	0.412			
Serum creatinine at 1 year	**1.007**	**1.003–1.012**	**0.001**	**1.009**	**1.004–1.015**	**<0.0005**
DCs[e]						
BDCA-1	**1.064**	**1.007–1.121**	**0.027**			
BDCA-2	1.044	0.999–1.088	0.056			
DC-SIGN	**1.039**	**1.003–1.074**	**0.034**	**1.052**	**1.012–1.093**	**0.010**
DC-LAMP	1.004	0.945–1.063	0.888			

Abbreviations: BDCA-1, blood dendritic cell antigen 1; BDCA-2, blood dendritic cell antigen 2; CI, confidence interval; DC, dendritic cell; DC-LAMP, DC-lysosomal-associated membrane protein; DC-SIGN, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin; eGFR, estimated glomerular filtration rate; HR, hazard ratio; PRA, panel reactive antibodies; PTC, peritubular capillaries.
[a]Graft outcome is defined as return to dialysis or doubling of serum creatinine relative to the values after 1 year posttransplantation.
[b]Score t2–t3 vs. t0–t1.
[c]Reference category is: No infiltration.
[d]Reference category is: PTC 0.
[e]HRs and 95% confidence intervals are calculated per 10^4 pixels.
Significant *P*-values are given in bold.

The maturation status of DCs is important in determining the outcome of T-cell activation.[44] Pretransplant biopsies showed almost a complete absence of mature DCs, reflecting the state of peripheral tolerance in the normal kidney.[45] Upon exposure to activation signals, including transplant-associated danger signals, DCs undergo a maturation program and migrate toward local lymph nodes where T-cell activation takes place.[15,16,44,45] Importantly, high numbers of mature DCs were detected in the rejected kidneys, indicative of local immunogenic conditions. Interestingly, mature DCs were most frequently observed in clusters of lymphocytic infiltrate and the amount of mature DCs correlated strongly with the Banff score for immunologic infiltrate. The presence of mature DCs in close proximity with T cells suggests that T cells are locally activated within the graft at the time of acute rejection. *De novo* formation of lymphoid-like structures in peripheral tissue is known as lymphoid neogenesis, and their presence has been described in chronic inflammation[46] and allografts, containing

DCs, T cells, and B cells.[47–49] Despite the strength of mature DCs to activate T cells, we did not observe a negative influence of the presence of mature DCs on long-term kidney function. The most likely explanation is that the presence of mature DCs represents a T-cell-mediated response that is effectively treated with current rejection treatments. A similar argument of effective treatment could explain the fact that the mere presence of tubulitis did not negatively affect long-term graft function (Table 6). After elimination of tubulitis, HR for DC-SIGN was 1.046 (1.008–1.085). After elimination of DC-SIGN, HR for tubulitis rose to 0.450 (0.165–1.226). HR for serum creatinine was nearly constant after these manipulations. However, we could not find any cases where prediction changed drastically after comparing different models. After removal of cases with more or less remarkable change in prediction, the HR values for tubulitis varied from 0.321 (0.115–0.897) to 0.453 (0.166–1.236) (for different combinations of case exclusions). For DC-SIGN, the HR values varied from 1.047 (1.006–1.088) to 1.086 (1.032–1.139). Therefore, we feel that it is unlikely that the reported effect is caused by outliers in the analysis.

Several factors have been proposed to contribute to the development of acute rejection, including ischemia–reperfusion injury resulting in DGF.[50] We found a correlation between the presence of pDCs and DC-SIGN$^+$ mDCs and the occurrence of DGF in patients receiving a deceased donor kidney. This is in line with previous studies that found an increase in DCs during DGF, induced by ischemia–reperfusion injury.[33,51] Resident DCs, present in pretransplant biopsies, function as passenger leukocytes at the time of transplantation and have been reported to produce large amounts of the proinflammatory cytokine, tumor necrosis factor, as a consequence of ischemia–reperfusion injury,[52,53] resulting in increased DC recruitment.[33,51] The production of tumor necrosis factor by DCs in the kidney is associated with the onset and amplification of an inflammatory response, which can lead to acute allograft rejection, but also with induction of renal epithelial damage and tubular atrophy.[53–55] This is in line with the correlation we found for the presence of both mDC subsets and pDCs with the Banff score for chronic changes, particularly interstitial fibrosis and tubular atrophy.

Diffuse C4d staining, as a possible sign of humoral rejection, was observed in 13 biopsies (13.4%). Unfortunately, in this retrospective study, we do not have serum available and no information on donor-specific antibodies. Therefore, we cannot firmly conclude on the contribution of humoral rejection, although the percentage of C4d positivity is much lower than the steroid resistance (48%).

There was no significant effect of the introduction of C4d or the other new results of the pathological scoring into the multivariable model and did not change the conclusion on the predictive role of DC for long-term graft function (Tables 5 and 6).

Acute rejection has been identified as the dominant risk factor for the development of what was initially described as chronic allograft nephropathy.[56,57] Early development of tubular atrophy and interstitial fibrosis are the first indications of the development of chronic allograft nephropathy. It was shown in protocol biopsies that deterioration of kidney function after kidney transplantation is preceded by chronic allograft nephropathy.[58,59] In addition, prognosis is negatively affected by the presence of infiltrate in areas of fibrosis.[60,61] The progressive decline in kidney function is obviously not attributable to one specific cause, but the severity and quality of acute rejection, including the composition of the infiltrate, will certainly have an important role. As expected, we found a very strong correlation of all DC subsets with the total inflammation score of the Banff 07 classification, an important predictor of outcome.[62] However, only for the density of mDC subsets at the time of acute rejection we found an independent predictive value for the loss of renal function in the long term. In our cohort of acute rejection, we observed diffuse C4d staining, as a possible sign of humoral rejection, in 13 biopsies (13.4%). However, as in this retrospective study we have no serum available and no information on donor specific antibodies, we cannot make firm statements on the occurrence of acute antibody-mediated rejection. Nevertheless, in this study, we did not observe significant effects of C4d positivity on long-term graft function.

The presence of mDCs was associated with early chronic changes, defined by interstitial fibrosis and tubular atrophy, within the graft during acute rejection. This suggests a role for this DC subset in the pathogenesis of interstitial fibrosis and tubular atrophy, implying that mDCs could be a target for future interventional therapy. On the other hand, it cannot be excluded that mDCs are the consequence rather than the cause of injury, and further studies are needed to determine the biological role of renal mDCs that are present during acute rejection. Nevertheless, the present study identified DC-SIGN as a novel prognostic factor in acute renal allograft rejection.

In conclusion, we showed for the first time that acute renal allograft rejection is characterized by the influx of both mDCs and pDCs, which is associated with local injury of the graft. The presence of mature DCs in close proximity with T cells within the kidney during acute rejection suggests local activation of the T cells within the graft. High numbers of mDCs at the time of acute rejection were identified as risk factors for long-term allograft dysfunction. Therefore, interventions in DC recruitment toward the kidney during acute allograft rejection may be a novel option to prevent acute rejection and improve long-term allograft survival. In addition, it expands our knowledge of the complex mechanism of acute allograft rejection and progressive loss of renal function.

MATERIALS AND METHODS

Patient characteristics and tissue specimen

We selected biopsies of patients transplanted between 1995 and 2005 at Leiden University Medical Center, who experienced an acute renal allograft rejection. Only biopsies obtained before treatment of a first acute rejection episode occurring within 6 months after

transplantation with available frozen material were included. Biopsies were taken on clinical indication (increase of serum creatinine >10% relative to baseline). For the present study, histological slides were reevaluated by two independent pathologists according to the Banff 07 criteria.[63] A total of 102 frozen biopsies were available for analysis. Acute rejection occurred at a median of 15 days (range: 3–178 days) after implantation. Of these 102 rejection biopsies, 78 corresponding pretransplant biopsies obtained before reperfusion at the time of transplantation were available. Immunosuppressive therapy consisted of corticosteroids, a calcineurin inhibitor, mycophenolate mofetil, and, in more recent years, an interleukin-2 receptor blocker. Recipient, donor, and transplant characteristics are summarized in Table 1. Renal function was estimated (estimated glomerular filtration rate) using the four-value Modification of Diet in Renal Disease (MDRD) formula.[64]

After the first year posttransplantation, 95 patients were still in the study. Graft loss or significant loss of renal function (18 patients), or death with a functioning graft (17 patients), occurred in 36.8% of patients. Graft loss/significant loss of function was caused by acute/chronic rejection ($n=14$), recurrence of original disease ($n=2$), or by an unknown cause ($n=2$).

Immunohistochemical staining

The presence of DCs during acute rejection was determined on 4 μm frozen tissue sections. After slides were fixed in ice-cold acetone, endogenous peroxidases were blocked with 0.1% NaN_3 and 0.1% H_2O_2 in phosphate-buffered saline for 30 min, blocked for 45 min with 1% bovine serum albumin and 5% normal human serum in phosphate-buffered saline, and subsequently incubated overnight with the following primary antibodies, all of mouse origin: BDCA-1, BDCA-2 (Miltenyi Biotec, Utrecht, The Netherlands); DC-SIGN (R&D Systems, Abingdon, UK); and DC-LAMP (Immunotech, Marseille, France). Primary antibodies were detected by incubation with a horseradish peroxidase-labeled goat-anti-mouse Ig antibody (Dako, Glostrup, Denmark) diluted in phosphate-buffered saline for 60 min, followed by incubation with Tyramide-fluorescein isothiocyanate (Tyramide-FITC) in tyramide buffer (1 mol/l Tris, 50 mmol/l imidazole, pH 8.8) for 20 min. The Tyramide-FITC signal was detected by a horseradish peroxidase-labeled rabbit anti-FITC antibody (Abcam, Cambridge, UK) for 60 min, followed by development in 3,3′-diaminobenzidine (Sigma-Aldrich, St Louis, MO). Sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany) and mounted with imsol (Klinipath, Duiven, The Netherlands) and entellan (Merck). All antibodies were diluted in phosphate-buffered saline containing 1% bovine serum albumin and 1% normal human serum.

Quantification of DCs in renal tissue

Numbers of DCs in the rejection tissue were determined by standardized measurement of positively stained area. For this analysis, pictures were taken from the complete biopsy with a ×100 magnification. Positively stained area was measured using ImageJ, a digital image analysis program (http://rsb.info.nih.gov/ij/). For the current analysis, a macro was developed, which splits the colored signal into a red, green, and blue channel. The amount of positive pixels measured in the blue channel was used for further analysis.

Statistical analysis

For the description of the data scale, variables are shown as means ± s.d. and nominal variables as numbers and percentages (%). Differences between pre- and post-transplantation DC subsets were analyzed using the Wilcoxon signed-rank test. For correlations between DC subsets in rejection tissue, Spearman's rho was calculated.

We observed that BDCA-1, BDCA-2, and DC-LAMP followed a γ-distribution. Uni- and multi-variable generalized linear modeling was used to build the model for predicting the amount of a single marker (counted as pixels) in a biopsy specimen. Simple inverse link function was used for the dependent variable and predictor variables were all analyzed as dichotomies. Only main relationships were studied. Parameter estimates were calculated using the maximum likelihood method. Because no apparent order was assumed for the predictor variables, type III model effects analysis was performed using the likelihood ratio statistics. For adjustment of multiple comparisons, sequential Sidak method was used. Because DC-SIGN showed an approximately normal distribution, generalized linear modeling using identity link function was performed. All other specifications were as mentioned above. The results are given as *P*-values for model effects, and the model-predicted means and 95% confidence intervals.

Uni- and multi-variable logistic regression analysis was performed to predict the relationship between single markers and inferior outcome at 1 year after transplantation, defined as allograft loss or an estimated glomerular filtration rate of <50 ml/min. The results are given as *P*-values and odds ratios with their 95% confidence intervals. Inferior outcome beyond the first year was defined as return to dialysis and/or doubling of serum creatinine relative to the 1-year value. Uni- and multi-variable analysis using Cox proportional hazards regression was performed to examine the effect of single markers on graft function/loss. These results are given as *P*-values and HRs with their 95% confidence intervals. Patient and graft survival at 1 and 5 years were estimated using Kaplan–Meier product limit method. The *P*-values of <0.05 were considered statistically significant. All analyses were performed with SPSS 17.0 for Windows (SPSS, Chicago, IL).

DISCLOSURE

All the authors declared no competing interests.

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