Macrophage density in early surveillance biopsies predicts future renal transplant function

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Inflammation impairs renal allograft survival but is difficult to quantify by eye at low densities. Here we measured leukocyte abundance in early surveillance biopsies by digital image analysis to test for a role of chemokine receptor genotypes and analyze the predictive value of leukocyte subsets to allograft function. In six-week surveillance biopsies, T-cell (CD3), B-cell (CD20), macrophage (CD68), and dendritic cell (CD209) densities were assessed in whole slide scans. Renal cortical CD3, CD20, and CD68 were significantly higher in histologic rejection. The CCR2 V64I genotype was associated with lower CD3 and CD209 densities. Above-median CD68 density was significantly associated with lower combined patient and graft survival with a hazard ratio of 3.5 (95% confidence interval 1.1-11.0). Both CD20 and CD68 densities inversely correlated with estimated glomerular filtration rate (eGFR) four years after transplantation. Additionally, CD68 correlated with eGFR loss. Among histological measurements including a complete Banff classification, only CD68 density was a significant predictor of an eGFR under 30ml/min after four years (odds ratio 7.4, 1.8-31.0) and part of the best eGFR prediction set in a multivariable linear regression analysis of multiple clinical and pathologic parameters. In a second independent cohort, the original CD68 median maintained its discriminative power for survival and eGFR. Thus, digital high-resolution assessment of CD68$^+$ leukocyte infiltration significantly improves prognostic value of early renal transplant biopsies.

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Kidney transplantation remains the most effective currently available form of renal replacement therapy, but graft survival is limited.[1,2] Risk factors for graft deterioration include infiltration by inflammatory cells.[3–5] However, in early 6-week surveillance biopsies, the Banff total inflammation score did not correlate with inflammatory gene mRNA expression, and only gene expression was associated with graft function,[6] whereas in another study,[7] inflammation according to the Banff criteria in early biopsies was associated with donor-specific antibody development at 1 year. Furthermore, in a number of studies in older grafts, the total amount of renal allograft inflammation in surveillance biopsies was a significant predictor of deterioration and failure.[8–13] Whether a low level of histologic inflammation in early surveillance biopsy specimens predicts functional outcome has not been reported.

Studies addressing leukocyte subtypes in nonrejecting renal allografts revealed increases in CD3$^+$ T cells, CD20$^+$ B cells, and CD68$^+$ macrophages in inflammatory infiltrates.[11,12] Increased B-cell density was associated with fibrosis and decreased function in surveillance biopsies from the first year after transplantation.[11] However, most B-cell accumulation seems to occur at later stages.[14] Macrophage marker CD68$^+$ myeloid cells have been detected in renal allografts during rejection and associated with functional decline.[15–18] In surveillance biopsies performed 1 year after transplantation, CD68$^+$ cells correlated with fibrosis and loss of function.[19] A recent study in for-indication biopsies suggests that in grafts with high levels of inflammation and a positive total inflammation score, myeloid dendritic cells characterized by CD209 in addition to CD68 may be superior indicators of graft prognosis.[20] There is no currently available report of a systematic assessment as to whether the amount of any of these cell types in early surveillance biopsies is relevant for prognosis.

The kidney contains a complex array of phagocytes with macrophage and antigen-presenting cell characteristics.[21,22] Their recruitment is largely directed by chemokines and their cognate receptors.[23] Data mainly from mouse models indicate that both CCR2 and CX3CR1 contribute to phagocyte accumulation in renal inflammation.[24,25] The CCR2 ligand CCL2 (MCP-1) was highly elevated during renal graft rejection and has been proposed as therapeutic

target.[2,26–28] Its V64I mutation has been associated with a positive,[29–32] negative,[33] or no change in outcome after renal transplantation.[34,35] CX3CR1 is expressed on renal phagocytes[36,37] and upregulated during fibrosis and renal graft rejection.[36,38] Although small studies on CX3CR1 polymorphisms did not show differences in renal graft survival,[29,39] a recent study indicated that the V249I mutation protects from acute kidney injury in sepsis.[40] Functional studies demonstrate enhanced adhesiveness for the V249I mutation.[40,41] The role of CCR2 and CX3CR1 genotypes in renal graft leukocyte accumulation has not been evaluated.

Manual assessment of cellular infiltrates in renal allograft biopsies is time-consuming, and quantification is difficult at low cell densities, increasing the probability of inter- and intraobserver variation.[42,43] For research purposes, high-resolution determination of the inflammatory infiltrate (e.g., by gene array analysis) may be preferable. However, less costly and more widely available technology would be helpful in clinical practice. A number of digital imaging tools have been introduced into pathology.[44] These require validation for each specific setting, not only in relation to current histopathologic standards, but also clinically meaningful outcomes.[45–47] Such studies on leukocyte densities in the renal allograft are not available so far.

The aim of this project was to assess digital quantification of T-cell, B-cell, macrophage, and dendritic cell infiltration in early surveillance biopsy samples of renal allografts compared with conventional diagnosis of inflammation according to the Banff criteria. We investigated the role of common human chemokine receptor polymorphisms in their accumulation and the relevance of cell densities for graft functional development.

RESULTS

Density of innate and adaptive leukocyte markers is significantly increased in renal allografts with rejection

Clinical characteristics of the 67 transplant recipients are given in Table 1. According to the revised Banff criteria, a rejection was present in 16% of grafts, mostly Banff category 3 borderline rejections (Supplementary Table S1). Whole-slide scans were annotated for renal cortex, medulla, and extrarenal tissues (Supplementary Figure S1). T-cell (CD3), B-cell (CD20), macrophage (CD68), and dendritic cell (CD209 = DC-SIGN) marker densities were quantified in whole specimens (methods, Supplementary Figure S1). We first analyzed whether leukocyte densities were associated with rejection. Indeed, renal cortical and medullary CD3, CD20, and CD68 positive infiltrates were significantly greater in grafts with rejection (Figure 1, Table 2), whereas CD209 showed a nonsignificant trend toward a decrease.

CD3, CD20, and CD68 densities strongly positively correlated with each other (Table 2). In an analysis of rejecting and nonrejecting grafts separately, the association between T- (CD3) and B- (CD20) cell densities was significant for each group (Supplementary Figure S2). In nonrejecting grafts, positive associations of CD209 with CD20 and CD68 with CD3 and a negative association between CD68 and CD209 reached the significance level (Supplementary Figure S2).

These data demonstrate that a marked increase of B-cell, T-cell and macrophage density in renal allograft rejection can be captured by computer-assisted histologic analysis.

Table 1 | Characterization of the patient cohort

Recipient characteristics	
Age, yr	51.9 ± 1.8
Sex	26% (23) female
Caucasian ethnicity	99% (66)
BMI	25.2 ± 0.5
Dialysis before transplantation	91% (60)
Panel reactive antibodies at transplantation	10% (mean reactivity to 35% of panel)
Waiting time	871 ± 105 days
Underlying renal disease	
ADPKD	27% (18)
Hypertensive nephropathy	12% (8)
Diabetic nephropathy	9% (6)
Glomerulonephritis	25% (17)
Miscellaneous	12% (8)
Unknown	15% (10)
Graft characteristics	
Deceased donor	67% (45)
Cold ischemia time, h	9.5 ± 0.9
HLA mismatch (A,B,DR)	2.5 ± 0.2
Immunosuppression at first outpatient visit	
Baseline steroid dose	11.3 ± 0.8 mg/d
CNI (tacrolimus and cyclosporine A)	99% (66)
Mycophenolate	100% (67)
mTOR inhibitor	3% (2)
Graft function at first outpatient visit	
Serum creatinine, μmol/l	174 ± 10
Serum cystatin C, mg/l	1.9 ± 0.1
GFR CKD-EPI (creatinine), ml/min	42 ± 2
GFR CKD-EPI (cystatin C), ml/min	39 ± 2
Proteinuria, g/l	0.1 ± 0.02

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CNI, calcineurin inhibitor; GFR, glomerular filtration rate; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin.
Values shown are % (*N*) or mean ± SEM.

Role of chemokine receptor CCR2 and CX3CR1 genotypes for leukocyte density in kidney allografts

The role of human chemokine receptors CCR2 and CX3CR1 in renal allograft leukocyte accumulation was studied by comparing marker densities between the genotypes (Figure 2). All genotypes were in Hardy-Weinberg equilibrium and not significantly different from those of local healthy blood donors (Supplementary Table S2). The chemokine receptor CCR2 V64I allele in heterozygous and homozygous form was associated with significantly lower $CD3^+$ T-cell and $CD209^+$ dendritic–cell densities in the renal cortex (Figure 2a and d). Further analysis of grafts according to the CCR2 genotype revealed no difference in the frequency of rejection, and renal function did not differ