Clinical Significance of Macrophage Polarization in Antibody-Mediated Rejection of Renal Allograft

J. Kim[a], S.-E. Choi[a], B.J. Lim[a], Y.S. Kim[b], K.H. Huh[b], J. Lee[b], S.I. Kim[b], M.S. Kim[b], and H.J. Jeong[a,*]

[a]Department of Pathology, Yonsei University College of Medicine, Seoul, Korea; and [b]Department of Transplantation Surgery, Yonsei University College of Medicine, Seoul, Korea

ABSTRACT

Background. The significance of proinflammatory M1 (classically activated) and profibrotic M2 (alternatively activated) macrophages in antibody-mediated rejection (ABMR) after kidney transplantation has not been investigated.

Methods. Fifty-five biopsy-confirmed ABMR samples were stained with MRP 8/14 (a marker of M1 macrophages) and CD163 (a marker of M2 macrophages), and positive cells were counted in glomeruli and the tubulointerstitium, respectively. Patients were classified into M1 and M2 polarization groups according to the glomerular and tubulointerstitial M1:M2 ratio, and the results were compared with Banff scores, serum creatinine level, estimated glomerular filtration rate (eGFR), and graft survival.

Results. The glomerular M2 polarization group showed significantly higher chronic glomerulopathy scores, serum creatinine levels, and lower eGFR at the time of biopsy ($P = .019$ and $P = .015$, respectively) and 3-month postbiopsy ($P = .016$ and $P = .032$, respectively) than the M1 polarization group. The tubulointerstitial M2 polarization group had significantly lower glomerulitis, arteritis, peritubular capillaritis, and glomerulitis + peritubular capillaritis scores than the M1 polarization group, but there was no significant difference in renal function. Long-term graft survival was not associated with macrophage polarization.

Conclusion. Glomerular M2 polarization in ABMR biopsy samples is associated with chronic glomerular injury and poorer graft function, but without graft survival.

IT has been reported that macrophage infiltration in allograft kidneys is associated with antibody-mediated rejection (ABMR) and is a predictor of graft failure [1]. Two phenotypically and functionally distinct macrophage subsets have been described. Classically activated or M1 macrophages [2] are activated by interferon-γ and involved in proinflammatory action, whereas alternatively activated or M2 macrophages [3] are activated by interleukin-4 and participates in profibrotic and repair stages. The time sequence and clinical implications of the dominance of M1 or M2 infiltration (ie, macrophage polarization) have been studied in renal ischemia-reperfusion injury, various forms of glomerulonephritis, and diabetic nephropathy [4]. However, little is known about the significance of macrophage polarization in terms of inflammatory activity and predicting the prognosis of renal transplantation recipients. In this study, we evaluated macrophage polarization status in patients diagnosed with ABMR in renal allografts and investigated its clinical impact.

MATERIALS AND METHODS

Sixty-seven cases with a diagnosis of acute or chronic ABMR were retrieved from the registry of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. Of them, 12 cases were excluded because clinical data were not available or informed consent could not be obtained. Finally, 55 renal allograft biopsy samples from 55 patients, all indication biopsies, were included in the study, which was approved by the Institutional Review Board of Severance Hospital.

J. Kim and S.-E. Choi contributed equally to this article.

*Address correspondence to Hyeon Joo Jeong, Department of Pathology, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea. Tel: +82-2-2228-1766, Fax: +82-2-362-0860. E-mail: jeong10@yuhs.ac

0041-1345/18
https://doi.org/10.1016/j.transproceed.2018.02.037

Table 1. Baseline Patient Characteristics According to M1 and M2 Polarization

Variables	All Patients	Glomerular Macrophage Polarization			Tubulointerstitial Macrophage Polarization		
		M1 (n = 24)	M2 (n = 24)	*P* Value	M1 (n = 28)	M2 (n = 27)	*P* Value
Recipient age	38.6 ± 12.5	42.8 ± 12.4	34.0 ± 11.9	.016	44.1 ± 10.9	32.8 ± 11.5	<.001
No of HLA mismatches	3.1 ± 1.4	3.5 ± 1.5	2.7 ± 1.2	.042	3.5 ± 1.7	2.8 ± 1.1	.073
% PRA (class I)	20.5 ± 35.0	17.2 ± 27.5	8.4 ± 27.0	.385	15.3 ± 25.3	26.3 ± 43.5	.382
% PRA (class II)	15.0 ± 24.7	10.5 ± 18.4	12.4 ± 28.9	.832	13.3 ± 22.9	17.1 ± 27.3	.657
Maximum DSA MFI	8154.2 ± 6270.4	6501.9 ± 5688.8	8080.2 ± 5907.5	.429	7208.3 ± 6053.8	9055.0 ± 6486.4	.352
Duration from RT to biopsy (mo)	71.2 ± 75.6	58.8 ± 64.3	80.8 ± 82.0	.306	50.21 ± 54.6	92.9 ± 88.5	.038
Recipient sex, male:female	28:27	11:13	14:10	.386	14:14	14:13	.999
Living:deceased donor	46:9	21:3	20:4	.999	24:4	22:5	.729
LCM-CDC positive	3	2	0	.489	2	1	.999
ABO incompatibility	4	3	1	.609	4	0	.111
ABMR phenotype				.011			.702
Acute	38	21	13		20	18	
Chronic	17	3	11		8	9	
Concurrent ATCMR	17	1	6	.163	9	8	.840
DSA				.896			.999
Class I	10	5	5		5	5	
Class II	21	8	9		9	12	
Class I + II	8	2	4		4	4	
No DSA	10	5	4		5	5	

Abbreviations: ABMR, antibody-mediated rejection; ATCMR, acute T cell-mediated rejection; DSA, donor-specific antibody; LCM-CDC, lymphocyte crossmatching-complement-dependent cytotoxicity; MFI, mean fluorescence intensity; PRA, panel-reactive antibody; RT, renal transplantation.

The biopsies were graded according to the Banff scoring system of renal allograft pathology (cg, chronic glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, vascular fibrous intimal thickening; g, glomerulitis; i, inflammation; ptc, peritubular capillaritis; t, tubulitis; v, arteritis) [5]. In addition to direct immunofluorescent staining for immunoglobulins G, A, and M; complements C3, C4, C1q; and fibrinogen (DakoCytomation, Glostrup, Denmark), fresh frozen renal sections were stained for C4d using a monoclonal mouse anti-C4d antibody (Biogenesis, Poole, UK) and fluorescein isothiocyanate-labeled polyclonal rabbit anti-mouse immunoglobulin (DakoCytomation). To evaluate macrophage polarization, immunohistochemistry was performed on 3-μm paraffin-embedded tissue sections using Bond-III autostainer (Leica Microsystems, Ltd, Wetzlar, Germany). Endogenous peroxidase was inactivated using 0.3% hydrogen peroxidase for 10 minutes. Following retrieval by heat and pressure using the Bond Epitope Retrieval Solution 1/2 (ER1/ER2) at 98°C for 20 minutes, the sections were incubated for 30 minutes with mouse anti-human MRP 8/14 monoclonal antibody (dilution 1:200; clone 27E10, BMA Biomedicals, Augst, Switzerland) and mouse anti-human CD163 monoclonal antibody (dilution 1:150; clone 10D6, Leica Biosystems, Wetzlar, Germany) for M1 and M2 macrophages, respectively, and then secondary horseradish peroxidase-labeled mouse antibodies were applied. Diaminobenzidine was used as the chromogen.

The numbers of M1 and M2 macrophages were counted, and their ratios were separately calculated in glomeruli and the tubulointerstitium. The number of positive cells is expressed as cells per glomerulus. For the tubulointerstitium, the number of positively stained cells was counted in 10 consecutive high-powered fields in the cortex except for areas occupied by glomeruli and vessels and then converted to cells per square millimeter. Patients were divided into M1 and M2 polarization groups for glomeruli and tubulointerstitium according to the median M1:M2 ratio value in each histologic compartment. Macrophage polarization was correlated with Banff scores, graft function represented by serum creatinine levels and estimated glomerular filtration rate (eGFR) calculated by Modification of Diet in Renal Disease equation, and graft survival.

Statistical Analysis

Comparisons between 2 groups were performed using independent two-sample *t* tests for continuous variables and χ^2 or Fisher exact tests for categorical variables. Survival data were analyzed with Kaplan-Meier curves with log-rank tests and multivariable Cox hazards regression analysis.

All the tests were performed using SPSS version 23.0 (IBM, Armonk, NY, United States), and *P* values less than .05 were considered statistically significant.

RESULTS

The baseline demography of the patients is shown in Table 1. Recipients' age was significantly younger in glomerular and tubulointerstitial M2 polarization groups than M1 polarization counterparts ($P = .016$ and $P < .001$, respectively), whereas time to biopsy from transplantation was shorter in tubulointerstitial M1 polarization group ($P = .038$). In addition, glomerular M1 polarization group had higher numbers of HLA mismatch ($P = .042$) and was frequently associated with acute ABMR ($P = .011$). Other clinical characteristics were not significantly different in both groups, including percent panel-reactive antibodies, types of donor-specific antibodies, and treatment modalities.

Macrophages were identified in the glomeruli of 48 cases (87.3%). In these cases, the median numbers of glomerular M1 and M2 macrophages were 0.5 (range 0–13) and 2.2 (range 0–26), respectively, with a M1:M2 ratio of 0.17 (range 0–51.00). The median numbers of M1 and M2