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Original contribution

Endothelial activation, lymphangiogenesis, and humoral rejection of kidney transplants☆,☆☆

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Summary Antibody-mediated rejection (ABMR) is implicated in 45% of renal allograft failure and 57% of late allograft dysfunction. Peritubular capillary C4d is a specific but insensitive marker of ABMR. The 2013 Banff Conference ABMR revised criteria included C4d-negative ABMR with evidence of endothelial-antibody interaction. We hypothesized that endothelial activation and lymphangiogenesis are increased with C4d-negative ABMR and correlate with intragraft T-regulatory cells and T-helper 17. Seventy-four renal transplant biopsies were selected to include (*a*) ABMR with C4d Banff scores ≥2 (n = 35), (*b*) variable microvascular injury and C4d score 0-1 (n = 24), and (*c*) variable microvascular injury and C4d score = 0 (n = 15). Controls included normal preimplantation donor kidneys (n = 5). Immunohistochemistry for endothelial activation (P- and E-selectins [SEL]), lymphangiogenesis (D2-40), T-regulatory cells (FOXP3), and T-helper 17 (STAT3) was performed. Microvessel and inflammatory infiltrate density was assessed morphometrically in interstitium and peritubular capillaries. All transplants had significantly higher microvessel and lymph vessel density compared with normal. Increased expression of markers of endothelial activation predicted transplant glomerulopathy (P-SEL, $P = .003$). Increased P-SEL and D2-40 were associated with longer interval from transplant to biopsy ($P = .005$). All 3 markers were associated with increased interstitial fibrosis, tubular atrophy, and graft failure (P-SEL, $P < .001$; E-SEL, $P = .0011$; D2-40, $P = .012$). There was no association with the intragraft FOXP3/STAT3 ratio. We conclude that endothelial activation and lymphangiogenesis could represent a late response to injury leading to fibrosis and progression of kidney damage, and are independent of the intragraft FOXP3/STAT3 ratio. Our findings support the therapeutic potential of specifically targeting endothelial activation.

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1. Introduction

Antibody-mediated rejection (ABMR) has been implicated in 45% of renal allograft failure [1] and in 57% of new-onset late allograft dysfunction [2]. Endothelial injury is one of the diagnostic pathologic features of ABMR. Chronic ABMR (CAMR) is the sequela of repeated/subclinical ABMR episodes with persistent endothelial injury and repair, leading to chronic endothelial remodeling with lamellation and deposition of newly formed basement membranes in peritubular capillaries and glomeruli, causing allograft dysfunction. Several mechanisms may contribute to endothelial injury during ABMR: complement-dependent antibody binding to endothelial surface antigens, endothelial activation by antibody alone [3], and complement-independent mechanisms mediated by natural killer cells [4]. Complement-dependent endothelial injury is detected in renal biopsies by C4d deposition in peritubular capillaries [5]. However, C4d has low sensitivity in routine biopsies [6] and in ABO donor group–incompatible kidney transplants. Recent studies have shown that increased expression of endothelial cell transcripts predicted graft loss with more sensitivity than C4d alone [7,8].

Activated endothelial cells increase expression of cell adhesion molecules. Selectins, transmembrane glycoproteins that are part of the cell adhesion molecule superfamily, mediate adhesion and rolling of leukocytes to the activated endothelium, the first step in leukocyte recruitment, through the mechanisms of chemokine-activated adhesion and extravasation. P-selectin (P-SEL) is stored in α-granules of platelets and in Weibel-Palade bodies of endothelial cells, and is translocated to the cell surface of activated endothelial cells and platelets. E-selectin (E-SEL) is not expressed under baseline conditions, except in skin microvessels, but is rapidly induced by inflammatory cytokines.

An additional mechanism that may contribute to ABMR is lymphatic neoangiogenesis. Lymph vessel density, assessed by D2-40 immunohistochemistry and morphometric analysis, is increased in areas with cellular infiltrates in renal biopsies with acute cellular rejection [9]. Lymphangiogenesis enhanced immune responses in corneal transplant rejection [10], and inhibition of lymphangiogenesis prolonged allograft survival after islet transplantation [11]. However, whether posttransplant lymphangiogenesis is beneficial or detrimental to the graft or whether this contributes to ABMR is still a matter of debate.

The aims of our study were to evaluate pathogenic markers of endothelial activation and lymphangiogenesis during ABMR and CAMR, and to correlate such markers with the progression of renal damage following humoral rejection. We hypothesized that up-regulation of these markers is associated with pathophysiologic mechanisms of rejection, and with specific shifts in the intragraft T-helper phenotype (regulatory T cells [Tregs] versus T-helper 17 [Th17]). Furthermore, we evaluated the ability of these markers to predict graft loss.

Table 1 Clinical characteristics at time of renal biopsy

	C4d+DSA+ (n = 35)	C4d−DSA+ (n = 24)	Controls (n = 15)
Age (y) (range)	41 (28.0-48.5)	36.5 (27.5-46.2)	39 (30.5-50.5)
Sex, M/F (%)	20:15 (57:43)	18:6 (75:25)	9:6 (60:40)
Race, C/AA/H (%)	19/15/1 (54/43/3)	12/12/0 (50/50/0)	12/3/0 (80/20/0)
Donor type, D/LR/LU (%)	15/8/12 [a] (43/23/34)	14/3/7 [a] (58/12/29)	8/7/0 [a] (53/47/0)
HLA compatibility (incompatible/compatible)	1/26	2/26	0.6
HLA mismatches			
A/B/DR ≤ 3	16	16	9
A/B/DR > 3	19	8	6
Time between transplant and biopsy (d)	583	410	377
Indication for biopsy, n (%)			
Increased creatinine	21 (60)	20 (84)	10 (66)
Proteinuria	1 (3)	1 (4)	1 (7)
Increased creatinine and proteinuria	13 (37)	3 (12)	3 (20)
DGF	0	0	1 (7)
Creatinine (mg/dL)	4.49	5.78	4.10
Proteinuria, n (%)			
Negative	4 (11)	2 (8)	5 (33)
Subnephrotic	25 (72)	20 (84)	8 (53)
Nephrotic	4 (11)	0	1 (7)
Not available	2 (6)	2 (8)	1 (7)

Abbreviations: DSA, donor-specific antibody; M, male; F, female; C, Caucasian; AA, African American; H, Hispanic; D, deceased donor; LR, living related donor; LU, living unrelated donor; DGF, delayed graft function.

[a] $P = .037$.