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Original contribution

Immunohistochemical markers of tissue injury in biopsies with transplant glomerulitis☆,☆☆

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Summary Transplant glomerulitis is associated with suboptimal graft function. To understand its pathogenesis and to assess the parameters of potential prognostic value, we immunostained 25 paraffin-embedded allograft biopsies showing glomerulitis for markers of complement activation (C4d), cytotoxicity (Granzyme-B), apoptosis (Bcl-XL, Bcl-2, and Fas-L), and endothelial injury (von Willebrand factor). Staining was semiquantitatively assessed in different anatomical compartments, and comparison was made with 40 control allograft biopsies without glomerulitis. Biopsies with glomerulitis had more frequent incidence of "mixed" T-cell and antibody-mediated rejection compared with controls [8/25 (32%) versus 4/40 (10%), $P = .046$]. Furthermore, they had higher glomerular capillary-C4d scores (1.9 ± 1.1 versus 1.2 ± 1.2, $P = .015$), which tended to persist when biopsies showing transplant glomerulopathy were excluded. Higher glomerular capillary-C4d scores were observed in samples with versus without donor-specific antibody (2.5 ± 0.9 versus 1.2 ± 1.2, $P = .01$). Compared with controls, biopsies with glomerulitis had more intraglomerular (4.8 ± 4.5 versus 0.9± 0.8 cells/glomerulus, $P < .001$) and interstitial mainly peritubular capillary (6.1 ± 4.1 versus 3.2 ± 3.4 cells/hpf, $P = .002$) Granzyme-B^+ leukocytes. Higher mesangial–von Willebrand factor scores were noted in the glomerulitis group (1.8 ± 1.0 versus 0.8 ± 0.8, $P = .003$) and correlated with the percentage of inflamed glomeruli ($r = 0.54$, $P < .001$). Interstitial–von Willebrand factor was associated with a higher peritubular capillaritis score (interstitial–von Willebrand factor: 1.6 ± 1.2 versus no interstitial–von Willebrand factor: 0.6 ± 0.9, $P = .02$). Glomerular capillary–Bcl-XL was not associated with accommodation. Finally, no difference in Bcl-2 or Fas-L was observed upon comparing glomerulitis to controls. In conclusion, glomerular injury in transplant glomerulitis appears to be mediated by complement activation and cellular cytotoxicity. Mesangial– or interstitial–von Willebrand factor identified cases with more severe microcirculation injury.

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1. Introduction

Transplant glomerulitis is characterized by intracapillary glomerular leukocytic inflammation in kidney allografts. It can be observed in association with antibody-mediated rejection (AMR) and/or T-cell–mediated rejection (TCMR) [1-3]. The presence and grade of glomerulitis correlate with proteinuria, peritubular capillaritis, peritubular capillary (PTC)-C4d staining, detection of circulating donor-specific antibody (DSA), development of chronic transplant glomerulopathy (TGP) and suboptimal graft survival [3-4]. The pathophysiology of transplant glomerulitis is not well understood. Hence, we performed a morphology-based study to understand the pathogenesis and potential prognostic factors of transplant glomerulitis. We immunostained a series of allograft biopsies with transplant glomerulitis for markers of complement activation (C4d), cellular cytotoxicity (Granzyme-B), apoptosis (Bcl-XL, Bcl-2, Fas-L), and endothelial injury (von Willebrand factor, or VWF). We compared the pattern of staining of glomerulitis samples with that of allograft biopsies without glomerulitis.

2. Material and methods

2.1. Biopsy material

We retrieved 111 renal allograft biopsies from 111 patients who received kidney allografts at the University of Pittsburgh Medical Center with (n = 67) and without (n = 44) transplant glomerulitis. All specimens were "for cause biopsies."

The detailed clinical, laboratory, and histologic characteristics of these patients have been published elsewhere [4]. Data collection procedures were approved by the University of Pittsburgh Institutional Review Board (IRB protocol #9030095). In the current study, we have characterized the immunohistochemical profile of the biopsy material.

Transplant glomerulitis was graded using the Banff 97 grading system (g0-g3: 0%, <25%, 25-75%, and >75% of glomeruli being affected, respectively) [5]. Affected glomeruli were defined by the presence of 5 or more leukocytes/glomerulus on periodic acid–Schiff–stained slides [4,6]. We found the latter grading system to be superior to grading based on the most inflamed glomerulus or the presence of capillary loop occlusion by inflammation [4]. In addition to transplant glomerulitis, histologic parameters were evaluated according to the Banff 97 criteria for renal allograft pathology [5], and samples were classified as negative, suspicious, or diagnostic for acute AMR based on Banff 2001 criteria [7].

Immunohistochemical studies were performed on biopsies with Banff grade g2 or g3 glomerulitis and compared with those of biopsies without glomerulitis (g = 0). Grade g1 samples were not analyzed because we did not encounter significant differences in any of the assessed clinicopathologic parameters when comparing g1 biopsies to biopsies without glomerulitis (g0) [4]. All biopsies with g2 or g3 glomerulitis and all biopsies without glomerulitis were included when sufficient remnant tissue material was available for analysis [glomerulitis (n = 25): g2 (n = 17) and g3 (n = 8), no glomerulitis (n = 40)].

2.2. Clinical parameters

Basic demographic and clinical parameters were retrieved from electronic medical records. In particular, percent of enzyme-linked immunosorbent assay panel reactive antibody (ELISA PRA) and detection of DSA were documented. Immune cell function values (ng ATP/mL whole blood) assessed using the Cylex ImmuKnow (Columbia, MD) assay were also recorded. Graft function was followed for a median of 600 days (interquartile range, 280-1160 days). The primary outcome parameter was graft failure defined as return to dialysis or transplant nephrectomy. The secondary outcome parameter was the development of TGP (cg $\geq$ 1) on follow-up, if it was absent (cg = 0) on the index biopsy.

2.3. Immunohistochemical stains

A panel of immunoperoxidase antibodies was performed on 4-micron formalin-fixed, paraffin-embedded renal allograft biopsies:

1) C4d: antigen retrieval was performed using Cell Conditioner 1 (CC1; Ventana Medical Systems, Tucson, AZ). Polyclonal mouse primary antibody (ALPCO Diagnostics, Windham, NH) was used (1:50 dilution, 44-minute incubation).
2) Granzyme-B: antigen retrieval was performed using CC1. Monoclonal mouse primary antibody (clone#B-7; Dako, Carpinteria, CA) was used (1:25 dilution, 40-minute incubation).
3) Bcl-XL: antigen retrieval was performed using microwave and incubation in Protein Block (Dako #X0909). Monoclonal mouse primary antibody (Invitrogen, Camarillo, CA) was used (1:50 dilution, 1-hour incubation).
4) Bcl-2: antigen retrieval was performed using CC1. Monoclonal mouse primary antibody (clone #124; Ventana Medical Systems) was used (1:50 dilution, 1-hour incubation).
5) Fas-L: antigen retrieval was performed by steaming for 20 minutes in EDTA buffer (PH 8.0) and incubation in Protein Block (Dako #X0909). Polyclonal rabbit primary antibody (Thermo Scientific, Fremont, CA) was used (1:100 dilution, 1-hour incubation).
6) VWF (factor VIII–related antigen): antigen retrieval was performed using CC1. Polyclonal primary rabbit antibody (Dako) was used (1:500 dilution, 8-minute incubation).

Secondary antibodies: for C4d, Granzyme-B, Bcl-2, and VWF, affinity-purified biotinylated goat-antimouse