Patterns of C4d Staining in Patients with Lupus Nephritis

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KEY WORDS: lupus nephritis, complement, C4d

ABSTRACT

Background: C4d, a cleavage product of the activated complement component C4, has attained considerable significance in the last few years for its role in helping elucidate the pathophysiology of renal allograft rejection. This investigation attempts to study the patterns of C4d deposition in native kidneys of patients with lupus nephritis.

Methods: Renal biopsies from 16 patients with lupus nephritis were reviewed for C4d deposition in the glomeruli and peritubular capillaries. Renal involvement in these patients was classified histopathologically according to World Health Organization classification as class II (4 cases), class III (3 cases), class IV (8 cases), and class V (1 case).

Results: All 16 patients (100%) showed deposition of C4d in the glomerular structures, and one also had focal deposition of C4d in the peritubular capillaries.

Conclusion: In 2 previous studies that reported C4d staining characteristics in a total of 28 patients with lupus nephritis, all patients had C4d deposition in the glomeruli, and none had C4d staining in the peritubular capillaries. Although the findings of the current study confirm those of previous studies with regard to glomerular C4d deposition, the additional focal deposition of C4d in the peritubular capillaries (a marker of humorally mediated allograft rejection) in 1 patient is interesting.

INTRODUCTION

Serum complement split products (C4d and Bb) have been reported to be more accurate indicators of lupus activity than complement levels. Detection of urine C3d has been suggested as a way of identifying complement activation in lupus-associated renal disease. C4d levels in plasma correlate with the degree of disease activity. C4d is the cleavage product of the activated complement component C4, which is activated and degraded as part of the antibody-mediated classic complement pathway. The C4d thus generated binds covalently to endothelial cell surfaces and the extracellular matrix components of vascular basement membranes. In addition, C4d may also be generated by the antibody-independent mannose binding lectin pathway. In recent years, the immunohistochemical detection of C4d deposits in
renal allografts has led to significant changes in the understanding of the pathophysiology of renal transplant rejection. The deposition of C4d along the peritubular capillaries in renal allograft biopsies is considered a marker of humorally mediated allo-response.\textsuperscript{4,5} C4d positivity in the setting of significant allograft dysfunction has been reported to be an independent strong predictor of poor graft outcome.\textsuperscript{6} Glomerular C4d deposition has been reported in normal kidney tissue, lupus nephritis, IgA nephritis, membranous glomerulopathy, membranoproliferative glomerulonephritis, and in a case of pre-eclampsia.\textsuperscript{7-9} Kim and Jeong examined 21 cases of lupus nephritis, all of which showed diffuse granular deposition of C4d along the glomerular capillary loops.\textsuperscript{10} Zwirner et al reported glomerular C4d deposition in all 7 lupus nephritis cases that were examined.\textsuperscript{7} However, none of these 28 cases of lupus nephritis had C4d staining along the peritubular capillaries. Thus, C4d deposition along the peritubular capillaries has not been reported in normal kidneys or in native diseased kidneys, including lupus nephritis, anti-neutrophil cytoplasmic antibody disease, and anti-glomerular basement membrane antibody disease, to the authors’ knowledge.\textsuperscript{11}
Table 1. Demographic and Clinical Characteristics of Lupus Nephritis Cases

<table>
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<th>Gender</th>
<th>Race</th>
<th>HTN</th>
<th>DM</th>
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</table>

N/A = not available; - = Value not due at the time of preparation of the manuscript; 24h UP = 24-hour urine protein in mg; Cr at Bx = serum creatinine in mg/dL at the time of renal biopsy; Cr at 3-6m = serum creatinine in mg/dL at 3-6 months; Cr at 12m = serum creatinine in mg/dL at 12 months; Y = yes (presence); N = no (absence); M = male; F = female; HTN = hypertension; DM = diabetes mellitus; AI and CI = activity and chronicity indices, respectively; Class = histopathological class of lupus nephritis according to World Health Organization classification.

SUBJECTS AND METHODS

Twenty-one patients diagnosed with lupus nephritis between December 2001 and January 2004 were initially included in this study. Five had inadequate tissue with no glomeruli visualized in the specimens used for immunofluorescence, leaving 16 patients in the study (Table 1). The mean age (± SD) of the remaining 16 patients was 35.6 (± 10.65) years (range 19 to 53 years). Eleven of the 16 patients were female. Information about race was available for 11 patients, 6 of whom were African American and 5 were white. Information about comorbidities including hypertension and diabetes mellitus was available for 11 patients, 6 of whom had hypertension and 2 also had diabetes mellitus. The indication for renal biopsy was known in 10 patients, all of whom had proteinuria; 24-hour urine protein values were available for 8 patients, 5 of these had nephrotic range proteinuria. An additional 2 patients had evidence of proteinuria on urinalysis. Serum creatinine levels at the time of renal biopsy were available for 10 patients and 2 of these had serum levels greater than 1.5 mg/dL (132.6 µmol/L).

Using a dissecting microscope, each biopsy core was divided into 3 parts; 1 part was fixed in formalin for light microscopy, another part was fixed in glutaraldehyde for electron microscopy, and the remaining part was immersed in optimal cutting temperature compound (Sakina Finetechanical, Torrance, CA) for immunofluorescence staining. The formalin fixed sections were then stained with hematoxylin and eosin, periodic acid schiff, trichrome, and Jones methenamine silver. Fluorescein isothiocyanate (FITC)-conjugated polyclonal
antibodies, directed against human IgG, IgA, IgM, C3, C1q, albumin, and fibrinogen (DAKO, Glostrup, Denmark), were used to stain freshly frozen tissue sections. For the detection of C4d, indirect immunofluorescence was employed, using biotinylated mouse anti-human monoclonal antibody and a FITC-conjugated rabbit anti-mouse IgG antibody (Biogenesis, Poole, England). Cases of acute humorally mediated allograft rejection served as positive controls for C4d immunofluorescence staining.

The histopathologic renal involvement in the 16 cases was classified according to World Health Organization classification\(^\text{12}\) as class II (4 cases), class III (3 cases), class IV (8 cases), and class V (1 case) (Table 2). The 5 cases that were excluded from the study belonged to class II (2 cases) and class IV (3 cases). Using the method of Austin et al.,\(^\text{13}\) activity and chronicity indices were calculated. The intensities of immunofluorescence staining in the mesangium, peripheral capillary loops, and peritubular capillaries were reported on a scale ranging from – (negative staining) to 4+ (intense staining), Table 3.

**RESULTS**

Of the 16 cases of lupus nephritis included in this study, C4d was detected in the glomerular structures in all cases; one of which also had focal deposition of C4d in the peritubular capillaries (Figure 1). In the 16 cases with positive glomerular C4d staining, 13 had diffusely granular deposition of C4d along the peripheral capillary loops and mesangial areas (Figure 2); 2 cases had diffuse granular deposition of C4d in the tubular basement membrane and mesangial matrix but not in the peripheral capillary loops;
and 1 case had focal granular pattern of C4d deposition in the mesangium, peripheral capillaries, and tubular basement membrane. Mesangial IgG, IgA, and C3 deposition were found in all 16 cases (100%), C1q in 16/16 cases (100%), and IgM in 15/16 cases (94%), with varying degrees of staining intensity (Table 3). The solitary case with a focal pattern of C4d deposition along the peritubular capillaries also had a diffuse granular pattern of C4d deposition in the glomerular capillaries and mesangium; immunofluorescence was also positive for IgG, IgA, C1q, and C3, but was negative for IgM.

**DISCUSSION**

Two mechanisms have been suggested for glomerular C4d deposition in normal kidney tissue as well as diseased native kidneys. Markham et al suggest transient glomerular deposition and clearance of small amounts of immune complexes formed during natural antibody response mechanisms in vivo, which initiates the classic complement pathway resulting in generation and deposition of...
C4d. An alternative mechanism is the passive absorption of circulating C4d generated by systemic complement activation.

In lupus nephritis, glomerular C4d deposition is an expected finding and is thought to result from the immune complex mediated activation of the classical complement pathway. In regard to the glomerular deposition of C4d, the results of this study are very similar to those reported by 2 earlier studies. An interesting finding in this study’s series of patients was the deposition of C4d in a focal pattern along the peritubular capillaries in 1 patient. This contrasts with the authors’ existing knowledge that C4d deposition along the peritubular capillaries is a fairly specific marker of humorally mediated allograft rejection. This finding can have implications in the interpretation of C4d deposition along the peritubular capillaries of renal allografts from patients with a history of end stage renal disease secondary to lupus nephritis, in whom there have been reports of recurrence of lupus nephritis in up to 30% of the cases.

While acute or chronic humoral rejection is a known cause of C4d deposition along the peritubular capillaries, one can only speculate that a milder and more focal pattern of C4d staining may occur in cases with recurrent lupus nephritis in the allograft.

REFERENCES