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Glomerular C4d Deposition Indicates in situ Classic Complement Pathway Activation, but is not a Marker for Lupus Nephritis Activity

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This study was designed to evaluate whether glomerular C4d deposition may be a useful marker of lupus nephritis activity. Twenty-one patients diagnosed as having lupus nephritis (WHO class III: 4 cases; IV: 12 cases; V: 5 cases) were included. Mean patient age was 29.3 ± 13.5 years (range: 7-55 years). The presence and intensity of glomerular C4d deposition were compared with the corresponding histologic activity index for each case. Immunofluorescence for C4d showed diffusely granular staining along glomerular capillary loops, in all cases examined (1+, in 8 cases; 2+, in 7 cases; 3+, in 6cases). In eight cases, C4d deposition was found in the absence of capillary or mesangial C4 deposits. Moreover, the intensity of C4d deposits correlated with those of capillary IgG, IgA, C4, C1q, and fibrinogen deposits. However, C4d staining intensity did not correlate with the lupus nephritis activity index. Although glomerular capillary C4d deposition is a sensitive marker of classic complement pathway activation, it is not a sensitive marker for active lupus nephritis.

Key Words: Lupus nephritis, C4d, activity index(AI)

INTRODUCTION

Several clinical and histologic parameters have been reported to be related to disease activity in SLE patients. Some investigators have suggested that hypocomplementemia is a marker of disease activity.[1-6] Ricker et al.[6] reported that serum C3 and C4 levels correlate with the degree of SLE activity, whereas others have suggested that complement split products are more sensitive indicators of disease activity than the conventionally measured C3 and C4.[7-16] Muso et al.[17] reported that glomerular deposition of C3d in patients with lupus nephritis is significantly correlated with disease activity, and Senaldi et al.[9] demonstrated that serum C4d levels correlate with the degree of disease activity in lupus patients. However, the correlation between tissue C4d deposition and disease activity in lupus nephritis has not been studied. C4d is a degradation product of complement factor C4, which is typically activated during the classical complement cascade. The classic pathway of complement activation is characteristically initiated by conformational changes in immunoglobulin molecules after binding to specific antigens,[18,19] which is followed by the cleavage of most C4 domains into C4a and C4b, thus exposing a reactive short-lived thiolester group in C4b, which covalently binds to nearby molecules. C4b is then cleaved by C3bINA in the presence of C4b-binding protein into C4c and C4d,[20] and the latter covalently binds to endothelial surfaces and basement membranes via a proteolytically exposed thiolester group.[21] We questioned whether C4d deposition may be a useful marker of lupus nephritis activity by examining C4d immunofluorescence staining in renal biopsy tissues from 21 patients with lupus nephritis and by correlating the results obtained with clinical and histologic parameters.

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MATERIALS AND METHODS

Twenty-one patients diagnosed as having lupus nephritis in the Department of Pathology, Yonsei University College of Medicine between December 1999 and July 2002 were the subjects of this study. All patients met the American Rheumatism Association's revised criteria for the classification of this disease.[22] The indications of renal biopsy were; for evaluation of nephrotic syndrome with (2 patients) and without nephritic syndrome (19 patients). The mean age of the patients was 29.3 ± 13.5 years (range: 7 - 55 years), and 18 patients were female. Six patients had high serum creatinine levels (normal reference range: 0.5 - 1.4 mg/dl). Eight patients had nephrotic-range (> 3.0 g/day) proteinuria. Because two patients had not been previously checked for 24-hour urine protein excretion, a random urine protein/creatinine ratio was applied as a substitute.[23] At the time of biopsy, nineteen patients had low serum C3 levels (normal reference range: 90 - 180 mg/dl) at the time of biopsy and 17 patients had low C4 levels (normal reference range: 10 - 40 mg/dl) (Table 1). Biopsied cores were sectioned under a dissecting microscope into three parts: one was inserted into formalin for light microscopic examination, another into glutaldehyde for electron microscopic examination, and the remainder was embedded in optimal cutting temperature (OCT) compound

Table 1. Clinical and Histological Data of Lupus Nephritis

Case No.	Sex/age	Stage	Activity Index(AI)	Chronicity Index(CI)	Serum Cr (mg/dl)	Serum C3/C4 (mg/dl)	24hr-urinary protein excretion (mg/24hr)
1	F/15	IV	7	1	0.9	37.9/3.28	266.4
2	F/36	IV	16	2	1.6	20.7/2.95	1912
3	F/10	III	1	0	0.8	47.5/2.14	171
4	F/7	IV	6	1	0.5	112.0/17.80	438.7
5	F/36	IV	15	2	1.6	33.3/3.93	5302.8
6	M/13	V	6	0	0.8	18.4/1.62	858
7	F/29	IV	12	4	3.2	72.0/2.80	2039.5
8	F/11	III	7	2	0.6	98.7/21.7	1039.8
9	F/51	IV	4	2	1.2	17.3/3.67	11467.8
10	F/32	V	2	0	0.8	58.5/16.08	2090.9
11	M/24	III	8	0	0.7	30.0/4.24	2415.3
12	F/23	IV	7	0	0.6	34.9/4.31	1502
13	F/55	IV	16	2	1	21.9/2.45	7742.8
14	F/42	IV	11	2	1.8	20.2/5.07	5294.7
15	F/34	V	7	0	0.8	42.0/9.18	19200
16	F/40	IV	12	0	2	22.3/5.96	1082.0/43*
17	F/28	III	7	1	1.6	29.4/5.22	836.5/43*
18	F/29	IV	13	2	0.8	43.8/5.07	3951
19	F/43	V	7	1	0.9	58.0/16.20	2616
20	M/38	IV	12	1	1.8	20.5/3.14	915
21	F/20	V	9	2	0.8	33.6/6.00	816.4

*random urine protein/creatinine ratio.

(Miles Laboratories, Elkhart, IN, USA) for immunofluorescence (IF) stain. Formalin-fixed tissue sections were routinely stained with hematoxylin and eosin, periodic acid Shiff, trichrome, and methenamine silver. Fresh frozen tissue was stained with FITC-conjugated polyclonal antibodies directed against human IgG, IgM, IgA, C3, C4, C1q and fibrinogen (DAKO, Glostrup, Denmark). The deposition of C4d was detected by indirect immunofluorescence using a mouse anti-human monoclonal antibody (dilution, 1:50; Biogenesis, Poole, England, UK) and an FITC- conjugated rabbit anti-mouse immunoglobulin antibody (dilution, 1 : 40; DAKO). Twelve cases of essential hematuria, five cases of minimal change disease and eight cases of membranous nephropathy served as controls for C4d immunofluorescence staining.[24,25] Cases of lupus nephritis were classified as Class III (4 cases), Class IV (12 case), and Class V (5 cases) by light microscopy according to the WHO classification.[26] Activity and chronicity indices were calculated according to the method of Austin et al.[27,28] (Table 1). The intensities of immunofluorescence staining in the peripheral capillary wall and mesangium were classified according to the scale : -, +/-, 1+, 2+ and 3+.

Statistical analysis

To examine the validity of C4d deposition as a marker for active lupus nephritis, the patients were subgrouped according to lupus nephritis activity index (AI) as high or low about the value of 12.[27] Spearman's rank correlation non-parametric testing was used to correlate each subgroup with respect to the intensity of C4d immune deposition. Correlations were also searched for between C4d deposition and the depositions of other capillary and mesangial immunoglobulins and fibrinogen. Results were considered statistically significant when the corresponding p value was ≤ 0.05. Data were analyzed using dBSTAT 4.0 software (dBSTAT, 2002).

RESULTS

C4d, immunoglobulins, and fibrinogen deposition

Staining was absent or minimal in the mesangium and glomerular capillary wall in essential hematuria and minimal change disease.[24] In the case of membranous nephropathy, granular deposition of C4d was present along the capillary loop.[25] In all 21 cases of lupus nephritis, C4d was detected predominantly along the glomerular capillary loops with a coarsely granular pseudolinear staining pattern, involving the entire capillary circumference (1+, in 8 cases; 2+, in 7 cases; and 3+, in 6 cases) (Fig. 1). Mesangial staining of C4d was barely detectable in all of the 21 cases. Renal structures other than glomeruli were not stained. Mesangial immunoglobulin or

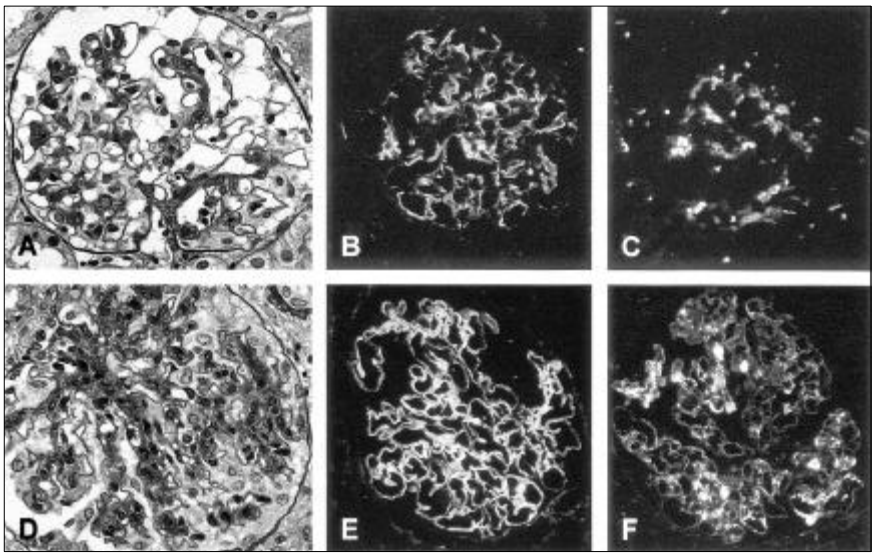

Fig. 1. A, B, C: A glomerulus (case 3) shows minimal mesangial proliferation (A, PAS × 400). Immunofluorescence staining in case 3 showing mild C4d deposition along the capillary loops (B, × 400) and moderate C3 deposition in the mesangium (C, × 400). D, E, F: A glomerulus (case 14) showing severe mesangial proliferation (D, PAS × 400). Immunofluorescence staining in case 14 showing moderate granular C4d immune deposition along the capillary loops (E, × 400). mild C4 deposition along the capillary loops with trace deposition in the mesangium (F, × 400).

complement deposition was present in all but three cases of membranous lupus nephritis. Capillary IgG deposition was present in 90.4%(19/21), IgA in 66.7%(14/21), IgM in 66.7%(14/21), C3 in 85.7%(18/21), C4 in 57.1%(12/21), and C1q in 61.9%(13/21) of the 21 cases, with varying degrees of staining intensity (Table 2). Intensities of the other capillary immune depositions increased as those of C4d deposition increased. Increased intensity of C4d deposition was significantly correlated with those of capillary IgG (r=0.644, p=0.002), capillary IgA (r=0.458, p=0.037), capillary C4 (r=0.742, p=0.0001), capillary C1q (r=0.550, p=0.001), and capillary fibrinogen (r=0.582, p=0.006) deposition.

Lupus nephritis activity index (AI), 24-hour urinary protein and C4d deposition

No significant correlation was found between the intensity of C4d immunofluorescence staining and a low (n=14) or high (n=7) lupus nephritis activity index (r=0.071, p=0.760)(Table 3) or any parameter of lupus nephritis AI (glomerular proliferation, leukocyte exudation, karyorrhexis/fibrinoid necrosis, hyaline deposits, or interstitial inflammation).

DISCUSSION

In the normal kidney, C4d was detected in the glomerular mesangium, in GBM and in the walls of some renal arterioles.[24] The patterns of positive glomerular staining reaction were granular and segmental in the mesangium and linear along the peripheral capillary loops. However, the other vascular compartments and the tubules were consistently negative.[24] No C4d was found in peritubular capillaries in normal or diseased kidneys except in transplanted kidneys.[29] Zwirner et al.[24] first described C4d immunostaining in

Table 2. Glomerular Immunofluorescence Findings

	Peripheral capillary wall								Mesangium						
Case	G	A	M	C3	C4	C1q	F	C4d	G	A	M	C3	C4	C1q	F
1	±	-	-	2+	-	±	-	1+	3+	1+	±	1+	-	2+	±
2	3+	3+	2+	3+	-	-	-	1+	3+	3+	1+	3+	-	-	-
3	-	±	-	-	-	-	-	1+	3+	2+	1+	2+	-	1+	-
4	±	-	-	±	-	-	-	1+	1+	-	±	±	-	±	1+
5	1+	-	1+	3+	-	1+	-	1+	3+	-	-	1+	-	-	-
6	1+	-	±	±	-	±	-	1+	3+	1+	2+	2+	-	3+	-
7	-	-	-	-	-	-	-	1+	3+	±	1+	1+	-	±	1+
8	2+	±	±	±	±	±	±	1+	-	-	-	-	-	-	-
9	1+	±	-	1+	±	-	-	2+	3+	3+	3+	2+	2+	3+	3+
10	2+	-	-	-	-	-	-	2+	1+	-	±	2+	-	±	-
11	3+	2+	1+	2+	2+	2+	2+	2+	3+	±	2+	1+	1+	2+	2+
12	3+	2+	2+	2+	1+	2+	1+	2+	3+	2+	2+	2+	1+	2+	1+
13	3+	±	±	±	1+	-	1+	2+	3+	3+	3+	3+	3+	3+	-
14	2+	2+	±	2+	±	1+	1+	2+	3+	±	1+	±	±	-	±
15	3+	2+	2+	1+	±	2+	2+	2+	-	-	-	-	-	-	1+
16	3+	1+	2+	3+	3+	3+	3+	3+	3+	3+	2+	3+	1+	2+	3+
17	3+	3+	2+	2+	3+	3+	3+	3+	3+	1+	2+	±	1+	3+	3+
18	1+	-	-	1+	-	-	-	3+	3+	1+	1+	2+	1+	1+	2+
19	3+	2+	1+	1+	1+	2+	-	3+	2+	1+	-	1+	±	±	-
20	3+	1+	1+	2+	2+	3+	3+	3+	3+	3+	3+	3+	1+	3+	3+
21	3+	2+	1+	3+	2+	1+	1+	3+	-	-	-	-	-	-	±

Table 3. Correlations between Glomerular C4d Deposition, Lupus Nephritis Activity Index(AI) and 24hr-urinary Protein Excretion by Spearman's Rank Correlation

	Subgroups	r	*p*
Activity Index	< 12 (N=14)		
	≥ 12 (N=7)	0.071	0.760
24hr-urinary protein excretion	< 3.0 g/day (N=13)		
	≥ 3.0g/day (N=8)	0.344	0.127

lupus nephritis as a strong, diffuse, granular pattern, and Senaldi et al.[9] reported that serum C4d level and C4d/C4 ratio provided a sensitive measurement of disease activity in SLE. In the present study, we evaluated the validity of C4d immunofluorescence staining as a useful marker of lupus nephritis activity. Glomerular epithelial crescents, fibrinoid necrosis, karyorrhexis, glomerular capillary deposits and the presence of vasculitis have been were reported to be histologic features of active lupus nephritis.[27,30] Austin et al.[27] proposed the lupus nephritis AI, which mostly focused on glomerular pathology. They reported that AI more strongly predicted renal failure than the individual active features of the biopsy specimen.[27] Abraham et al.[30] reported that a high AI was predictive of progression to end stage renal failure in patients with diffuse proliferative lupus nephritis. In the present study, no correlation was found between lupus nephritis AI and C4d glomerular capillary deposition. The intensity of C4d deposition correlated with those of capillary IgG, IgA, C4, C1q and fibrinogen. The coexpression of C4d, together with IgG and early classic complement components (C4 and C1q) suggests the activation of the classic complement pathway in the kidney.[18] Regardless of the presence or absence of capillary or mesangial immunoglobulins and fibrinogen deposition, all cases of lupus nephritis showed C4d deposition along the capillary loops. In our results, 12 cases (57.1%) and 13 cases (61.9%) showed C4 and C1q deposition along the glomerular capillary, respectively. These results indicate that C4d deposition seems to be a more sensitive marker of classic complement pathway activation than C4 or C1q deposition. However, C4d deposition does not seem to be a sensitive marker of present disease activity. The reason for C4d deposition predominantly along the capillary loops in lupus nephritis is unclear. More rapid degradation and clearance of C4d in the mesangium and a higher affinity of C4d to the peripheral capillary loops may be explanations. Mitjavila et al.[31] reported a strong correlation between the lupus nephritis AI with proteinuria. In our study, C4d immune deposition was found to be correlated neither with proteinuria (24hr-urinary protein excretion) (Table 3) nor with the serum C3 or C4 levels. In conclusion, capillary C4d deposition does not indicate present disease activity in lupus nephritis. However, it may provide useful evidence for prior activation of the classic complement pathway.

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