Membrane Attack Complex (MAC) Deposition in Renal Tubules is Associated with Interstitial Fibrosis/Tubular Atrophy and Proteinuria in Lupus Nephritis

Shudan Wang[1] MD, Ming Wu[2] MD, Luis Chiriboga[2] PHD, Beatrice Goilav[3] MD, Chaim Putterman[1,4] MD, Daniel Schwartz[5] MD, James Pullman[5] MD PHD, Anna Broder[1] MD MS, H. Michael Belmont[6] MD

[1] Division of Rheumatology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY [2] Department of Pathology, NYU Langone Health, New York, NY. [3]Division of Nephrology, Children's Hospital at Montefiore, Bronx, NY.
[4] Azrieli Faculty of Medicine, Bar-Ilan University, Zefat 52100, Israel. [5] Department of Pathology, Montefiore Medical Center, New York, NY [6] Division of Rheumatology, NYU Langone Health, New York, NY.

BACKGROUND & OBJECTIVE

Lupus nephritis (LN) is a heterogenous disease, in which 10 to 30% of patients progress to end stage renal disease (ESRD) within 10 years (1) despite standard treatment. Prior studies showed that interstitial fibrosis/tubular atrophy (IFTA) is associated with poor renal outcomes independent of glomerular damage and are reliable predictors of progression to ESRD (2). Tubulointerstitial injury in renal disease can be mediated by complement activation, specifically through the membrane attack complex (MAC, C5b to C9). Tubular deposition of the MAC was found to be associated with IFTA in animal studies (3). Only one prior study studied tubular MAC deposition with IFTA in LN patients but had not been reproduced in other LN population (4).

The aim of this study is to investigate the association of tubular MAC deposition with IFTA and proteinuria in LN.

It is timely to understand complement mediated tubulointerstitial injury as this could identify new avenues for LN treatment

METHODS

- This cross-sectional study used renal biopsies obtained from 30 SLE patients (July 2014 to July 2016) at NYU who met ACR or SLICC criteria were selected. ISN/RPS LN Class II, III, IV, V, III+V, or IV+V were included.
- Immunohistochemistry was performed on formalin fixed paraffin embedded renal tissue using unconjugated, murine anti-human complement C9, which is a part of the MAC.
- Positive control was C3 glomerulopathy. Negative control was normal tissue.
- Tubular basement membrane C9 staining was scored on intensity on a semiquantitative scale by three renal pathologists. Intensity was analyzed as negative (0) versus positive (1 to 3).
- Interstitial fibrosis tubular atrophy (IFTA) was categorized as categorized as <20% (none/mild) or ≥ 20% (moderate/severe)
- Clinical parameters including proteinuria assessed at time of biopsy.
- Fisher's exact test for categorical and t-test for continuous variables.

RESULTS

24 women, 6 men; mean age 32.9 ± 12.1 years; 4 Asians, 9 Blacks, 6 Caucasians and 11 Hispanics; Moderate SLEDAI activity with average SLEDAI 10.5 ± 3.8
Normal creatinine (1.1 ± 1.2 mg/dl); Low albumin (3.0 ± 0.7 g/dL)
Low complement and elevated dsDNA in 76.7% and 56.7%
11 out of 30 (36.7%) had nephrotic range proteinuria;
23 out of 30 (77%) patients had proliferative lupus nephritis.
3 out of 30 (10%) had moderate/severe IFTA
7 out of 30 (23%) biopsies stained positive for tubular C9

TABLE: Comparisons of Tubular C9+ and C9- renal biopsies

	Positive for Tubular C9 (n = 7)	Negative for Tubular C9 (n = 23)	P- Value
Clinical Parameters			
Age, median (IQR), years	36 (31-56)	27 (21-39)	0.20
Female sex, n (%)	5 (71%)	19 (83%)	0.60
Hispanic, n (%)	4 (57%)	7 (30%)	0.37
Hydroxychloroquine use, n (%)	4 (57%)	20 (87%)	0.12
Prednisone use, n (%)	2 (29%)	17 (74%)	0.07
SLEDAI-2K, median (IQR)	12 (10-12)	10 (6-12)	0.82
Systolic BP, median (IQR) mmHg	133 (113-150)	120 (110-130)	0.18
Laboratory Parameters			
eGFR, median (IQR), mL/min/1.73 m^2	57.9 (16.1-139.6)	118.2 (95.2-13.0)	0.17
Creatinine, median (IQR), mg/dL	1.6 (0.5-3)	0.7 (0.6-0.8)	0.23
Urine protein, median (IQR), g/24hr at biopsy	**6.2 (3.3- 13.1)**	**2.4 (1.3-4.6)**	**<0.01**
Albumin, mean ± SD, g/dL	2.5 ± 0.6	3.1 ± 0.7	0.05
Serum C3<70 mg/dL, n(%)	4 (57%)	16 (70%)	0.66
Serum C4<14 mg/dL, n(%)	3 (43%)	10 (44%)	0.18
Serum dsDNA ≤ 75 IU, n(%)	3 (43%)	10 (44%)	0.99
Renal Biopsy Light Microscopy			
Proliferative LN class (3, 4, 3+5, 4+5), n (%)	6 (85.7%)	17 (73.9%)	>0.99
Moderate NIH Chronicity Index (score 4-7), n (%)	3 (42.9%)	2 (8.7%)	0.07
Moderate NIH Activity Index (score 6-14), n (%)	2 (28.6%)	10 (43.5%)	0.67
Moderate to severe IFTA, n (%),	**3 (42.9%)**	**0 (0%)**	**0.01**
> 10% Global glomerulosclerosis, n (%)	5 (71.3%)	5 (21.7%)	0.03
Number of crescents	1 (0-2)	0 (0-2)	0.82
C9 deposition, non-tubular			
Glomerular (score >0), n (%)	3 (42.9%)	10 (43.5%)	>0.99
Arterioles/arteries (score >0), n (%)	6 (85.7%)	12 (52.2%)	0.19

RESULTS

At the time of renal biopsy, C9+ patients had significantly higher proteinuria, compared to C9- patients: median (interquartile range) 6.2g (3.3-13.1) vs. 2.4g (1.3-4.6), p<0.01. Tubular C9 deposition was associated with IFTA: 3 out of 7 (42.9%) had moderate/severe IFTA as compared to none in the C9- group, p=<0.01. Higher proportion of C9+ patients had moderate NIH Chronicity index: 3 out of 7 (42.9%) vs 2 out of 23 (8.7%) in the C9- group, p=0.07. There was no association between tubular C9 deposition with glomerular C9 deposition and proliferative LN class.

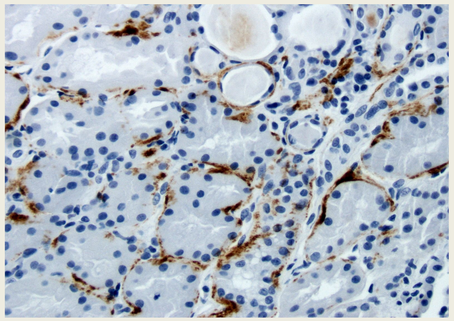

Figure:
Patient with Lupus Nephritis Class V, 2+ tubular C9 deposition and 30 to 40% IFTA.

CONCLUSION

Tubular MAC deposition was associated with proteinuria and IFTA, which are predictors of progression to ESRD. This suggest that the terminal complement pathway plays an important role in tubulointerstitial injury in LN independent of glomerular damage. Elucidating the role of the MAC in tubulointerstitial injury will aid in the identification of treatment targets in LN. This is timely with multiple complement targeting therapies in development.

REFERENCES

1. Hoover PJ, Costenbader KH. Insights into the epidemiology and management of lupus nephritis from the US rheumatologist's perspective. Kidney international. 2016;90(3):487-92.
2. Broder A, Mowrey WB, Khan HN, Jovanovic B, Londono-Jimenez A, Izmirly P, et al. Tubulointerstitial damage predicts end stage renal disease in lupus nephritis with preserved to moderately impaired renal function: A retrospective cohort study. Seminars in arthritis and rheumatism. 2018;47(4):545-51.
3. Nangaku M, Pippin J, Couser WG. Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. J Am Soc Nephrol. 1999;10(11):2323-31.
4. Wilson HR, Medjeral-Thomas NR, Gilmore AC, Trivedi P, Seyb K, Farzaneh-Far R, et al. Glomerular membrane attack complex is not a reliable marker of ongoing C5 activation in lupus nephritis. Kidney international. 2019;95(3):655-65.