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CD44 Expression in Serum and Kidney Correlates with Disease Activity in Lupus Nephritis

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INTRODUCTION

Lupus nephritis is characterized by a loss of immune tolerance to self-antigens leading to immune-mediated kidney injury (1). CD44 is a cell surface receptor for hyaluronan and has been implicated in tissue inflammation and fibrosis (2, 3). We investigated serum CD44 level and its renal expression in human and murine lupus nephritis, focusing on its role in renal fibrosis.

METHODS

- CD44 was measured in paired serum samples from 56 patients with biopsy-proven diffuse proliferative lupus nephritis during active disease and remission using a commercially available ELISA.
- Healthy subjects (n=36) and patients with non-lupus glomerular diseases (IgA nephropathy and diabetic nephropathy, n=40) were included as controls.
- Intra-renal expression of CD44 and hyaluronan was determined by cytochemical staining.
- Collagen deposition in kidney specimens was determined by Masson's trichrome staining.
- Mesangial cells were isolated from NZBWF1/J mice to investigate the mechanism of CD44 synthesis. Confluent, growth arrested cells of the 5-7 passage were stimulated with exogenous hyaluronan (0.05 - 5 μg/ml), IL-1β, IL-6 or TNF-α (10 ng/ml for all cytokines) for 24h, and CD44 synthesis assessed by Western blot analysis.

RESULTS

- Serum CD44 levels were significantly higher in patients with active lupus nephritis compared to levels in the same patients during remission, patients with non-lupus glomerular diseases, and healthy subjects ($P<0.001$ for all) (Figure 1A).
- Serum CD44 level correlated with anti-dsDNA antibody (r=0.43, $P<0.001$) (Figure 1B) and serum creatinine levels (r=0.46, $P<0.001$) (Figure 1C), and inversely correlated with C3 level (r=-0.56, $P<0.001$) in lupus nephritis patients (Figure 1D).

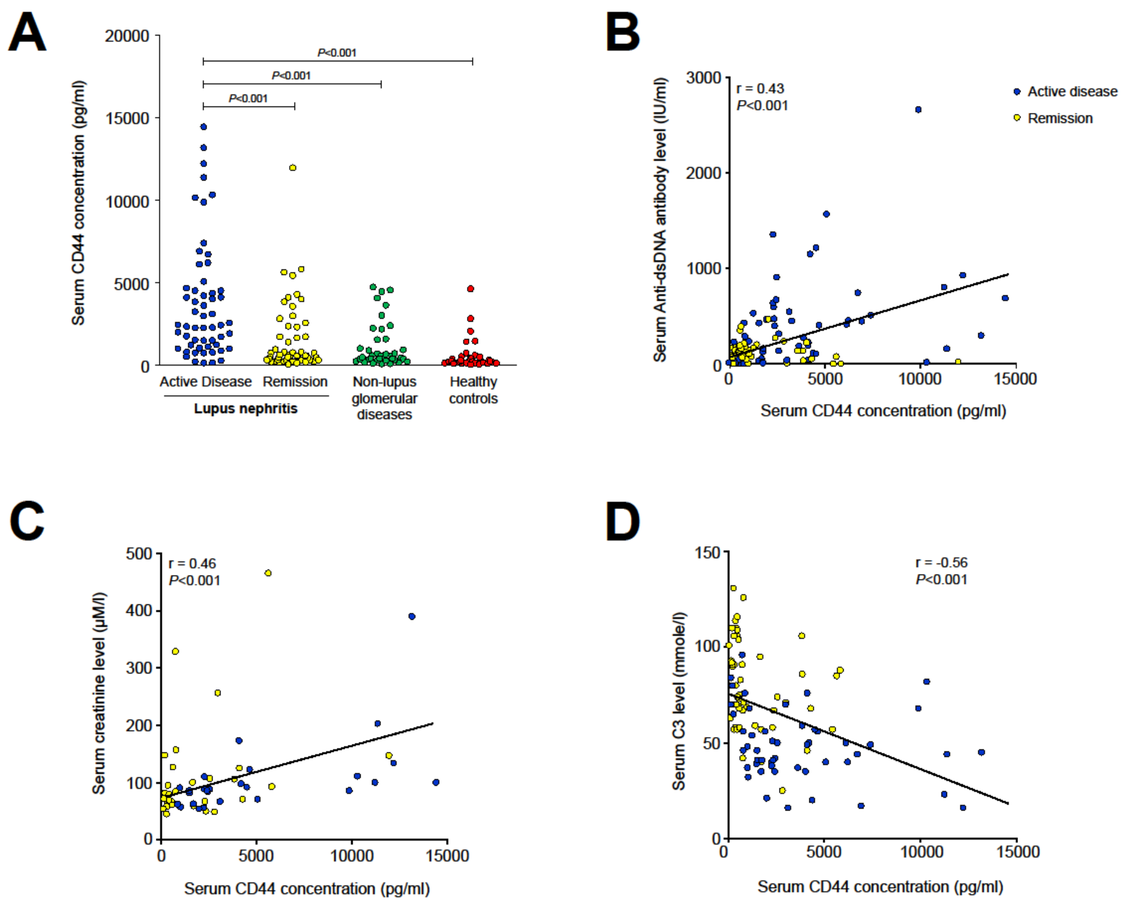

Figure 1. Comparison of serum CD44 levels in healthy controls, and patients with lupus nephritis or non-lupus glomerular diseases, and correlation of serum CD44 level with disease parameters in lupus nephritis patients

(A) CD44 levels were measured in serum samples from patients with lupus nephritis, patients with non-lupus glomerular diseases and healthy controls. Serum CD44 level correlated with (B) anti-dsDNA antibody and (C) creatinine levels, and (D) inversely correlated with C3 level.

- Kidney biopsies obtained from patients with active lupus nephritis showed strong CD44 staining in both resident renal cells and infiltrating cells in glomeruli and renal tubules, whereas normal kidney tissue showed weak CD44 expression (Figure 2).

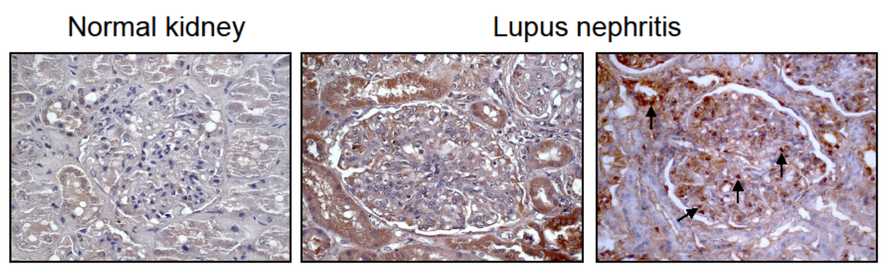

Figure 2. CD44 expression in human kidney specimens

Representative images of CD44 expression in normal kidney (left panel) and in renal biopsies from patients with active diffuse proliferative lupus nephritis (middle and right panels). Arrows show CD44 expression in infiltrating cells. Original magnification x400.

- Renal cortical CD44 gene and protein expression increased with progressive disease and was associated with increased hyaluronan expression and collagen deposition in NZBWF1/J mice (Figure 3).

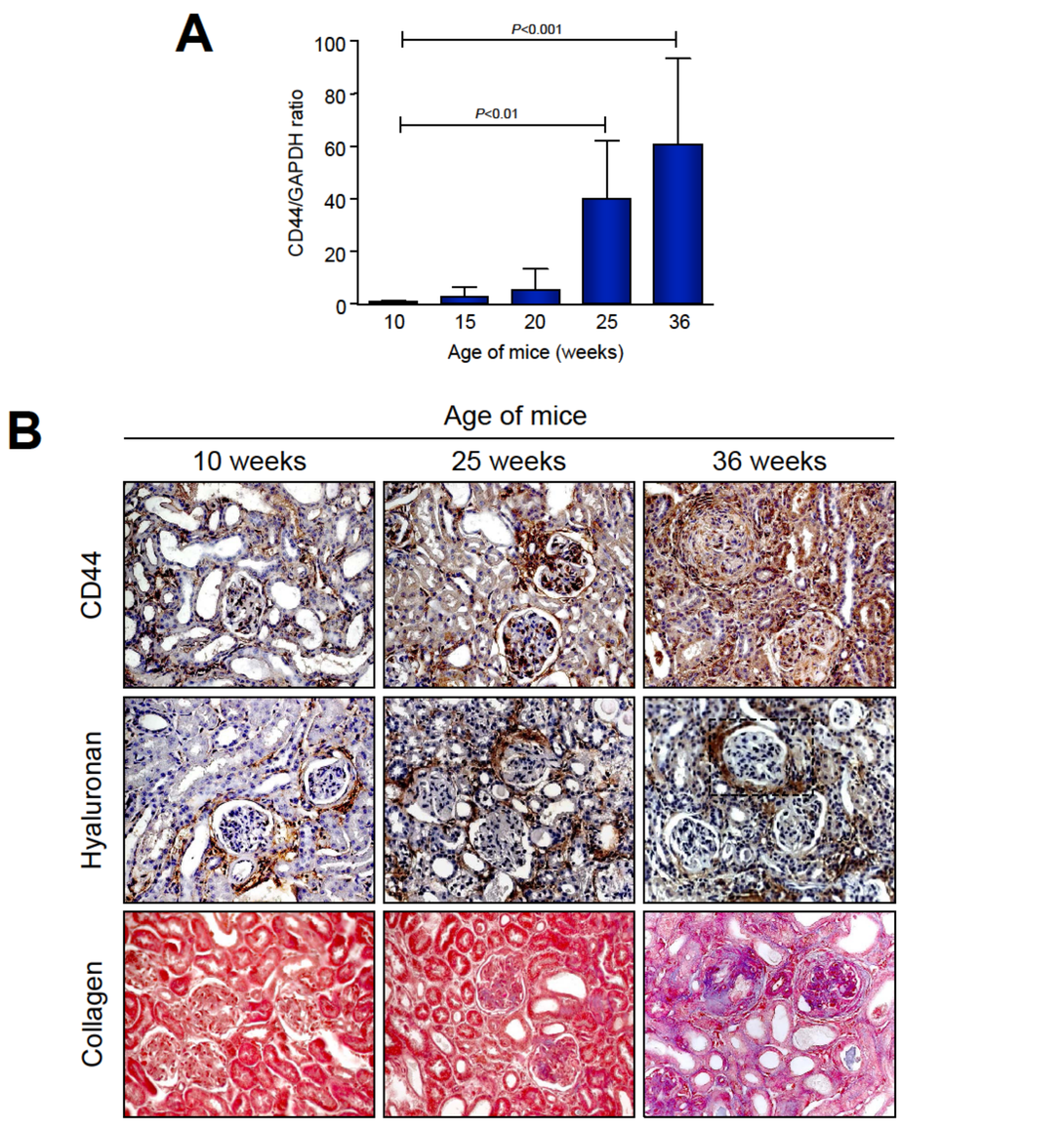

Figure 3. Intra-renal expression of CD44, hyaluronan and collagen during progressive lupus nephritis in NZBWF1/J mice

Representative images showing the expression of CD44 (upper panels) and hyaluronan (middle panels), and collagen deposition (blue coloration, lower panels) in kidney specimens from NZBWF1/J mice with progressive disease. Original magnification x400.

- In vitro experiments demonstrated that mesangial cells from NZBWF1/J mice constitutively synthesized CD44, and exogenous hyaluronan, IL-6, IL-1β, TNF-α induced CD44 synthesis (Figure 4).

Figure 4. Effect of exogenous hyaluronan, IL-6, IL-1β or TNF-α on CD44 synthesis in murine mesangial cells

Representative Western blots showing the effect of (A) exogenous hyaluronan and (B) IL-6, IL-1β and TNF-α on cell-associated CD44 synthesis in mesangial cells isolated from NZBWF1/J mice. SFM: serum free medium.

CONCLUSIONS

Our data suggest that CD44 is involved in the pathogenesis of renal inflammation and fibrosis in lupus nephritis.

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