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SHORT REPORT

Dissecting signalling pathways associated with intrarenal synthesis of complement components in lupus nephritis

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ABSTRACT

Lupus nephritis is one of the most common and serious complications of systemic lupus erythematosus, attributed to increased morbidity and mortality. The in situ deposition of intrarenal immune complexes promote the accumulation of inflammatory cells and cause kidney injury in lupus nephritis. Among potential sources of intrarenal complement deposits, the concept of intrarenal complement synthesis has been described more than three decades ago in experimental lupus nephritis. By using transcriptome datasets, we here identified accelerated intrarenal synthesis of distinct classical and alternative complement pathway components, most associated with impaired kidney function. Contrasting to this, no such induction of intrarenal complement synthesis was observed in disease controls, further supporting relevance of intrarenal complement synthesis especially in human lupus nephritis. Gene set enrichment identified that glomerular complement synthesis predominantly associated with interferon signalling and signalling by interleukins in human lupus nephritis, whereas tubulointerstitial complement synthesis with aberrant T-cell receptor signalling. Because the pathomechanistic involvement of complement system activation contributed to recent advances in targeted therapy in lupus nephritis, this study provides additional insights into signalling pathways associated with intrarenal synthesis of complement components in lupus nephritis that might be also affected by targeted therapy of the complement system.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lupus nephritis is one of the most common and serious complications of systemic lupus erythematosus, attributed to increased morbidity and mortality.
⇒ The in situ deposition of intrarenal immune complexes promote the accumulation of inflammatory cells and cause kidney injury in lupus nephritis.

WHAT THIS STUDY ADDS

⇒ We identified accelerated intrarenal synthesis of distinct classical and alternative complement pathway components, most associated with impaired kidney function.
⇒ Glomerular complement synthesis predominantly associated with interferon signalling and signalling by interleukins in human lupus nephritis, whereas tubulointerstitial complement synthesis with aberrant T-cell receptor signalling.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The pathomechanistic involvement of complement system activation contributed to recent advances in targeted therapy in lupus nephritis.
⇒ This study provides additional insights into signalling pathways associated with intrarenal synthesis of complement components in lupus nephritis.

INTRODUCTION

Lupus nephritis is one of the most common and serious complications of systemic lupus erythematosus (SLE).[1] Lupus nephritis is a major cause of kidney failure in patients with SLE, attributed to increased morbidity and mortality.[2] The in situ deposition of intrarenal immune complexes promote the accumulation of inflammatory cells and cause kidney injury.[3 4] Among them, complement system activation with decreased serum levels of complement C3 and C4 have been found in about 75% of patients with SLE with focal nephritis and 90% in patients with diffuse nephritis.[5] In addition, colocalisation of immunglobulin isotypes IgG, IgA and IgM along with C1q, C3 and C4 in the glomerular compartment is almost exclusively present in patients with lupus nephritis.[6] Among potential sources of intrarenal complement deposits, the concept of intrarenal complement synthesis has been described more than three decades ago in experimental lupus nephritis.[7] Complement system activation pathways, termed the classical, lectin and alternative merge into a final common pathway leading to assembly of the membrane attack complex. Previous studies have mainly focused on intrarenal synthesis of complement components C2, C3, factor B and C4 in lupus nephritis.[7] We here expand our

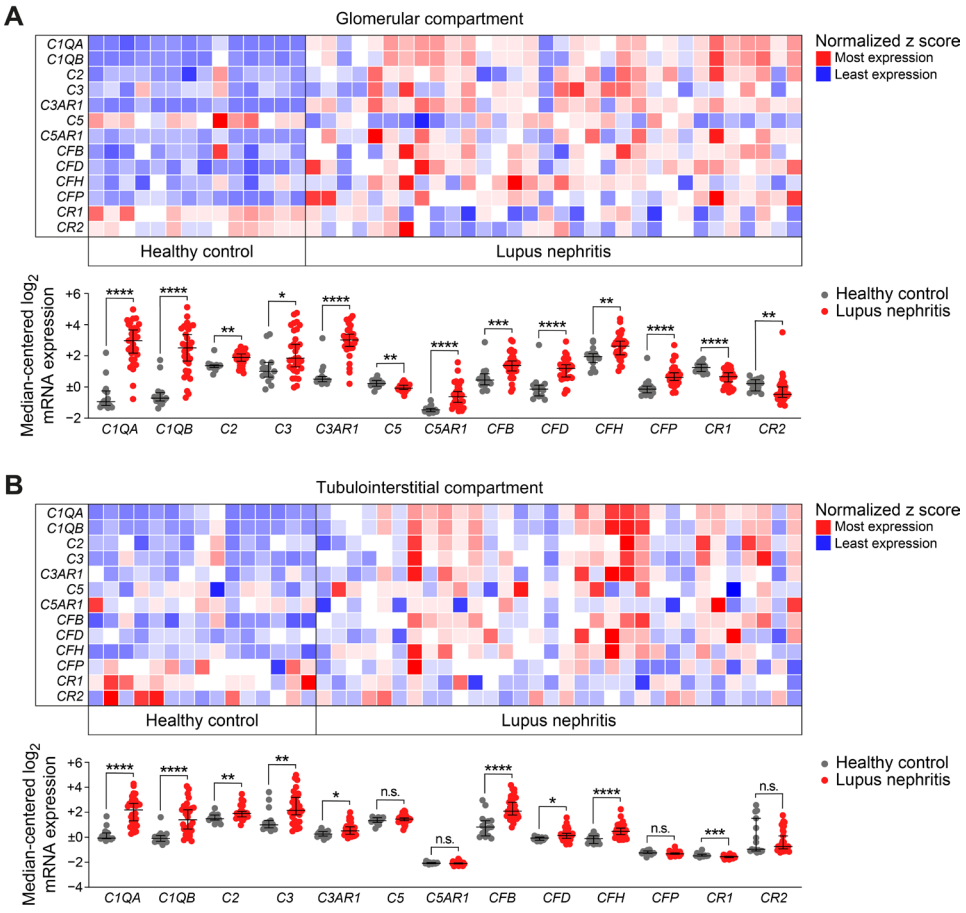

Figure 1 Accelerated intrarenal synthesis of distinct complement components in human lupus nephritis. (A,B) Glomerular and tubulointerstitial mRNA expression levels of indicated complement components in healthy controls and lupus nephritis. Median with 95% CI-centred $\log_2$ mRNA expression levels are shown, comparison of groups was performed using the Mann-Whitney U test to determine differences in medians unpaired t-test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n.s. not significant).

current knowledge about signalling pathways associated with intrarenal synthesis of complement components in human lupus nephritis.

METHODS

Data extraction from publicly available array datasets

Transcriptome array datasets were used from Nephroseq (www.nephroseq.org, June 2022, University of Michigan, Ann Arbor, Michigan, USA). Particularly, median-centred $\log_2$ mRNA expression levels (GSE32591, platform: Affymetrix Human Genome U133 Plus 2.0 Array, altCDF V.10) were extracted specifically from microdissected glomerular (14 healthy controls, 32 with lupus nephritis) and tubulointerstitial compartments (15 healthy controls, 32 with lupus nephritis, online supplemental tables 1,2).[8] For validation, median-centred $\log_2$ mRNA expression levels were extracted specifically from microdissected glomerular compartments (6 normal kidneys, 25 with lupus nephritis, online supplemental tables 3).[9] In addition, disease controls including hypertensive nephropathy, diabetic kidney disease and minimal change disease were also extracted.[10 11]

Gene set enrichment analysis

For gene set enrichment analysis, genes coexpressed with either glomerular or tubulointerstitial mRNA expression of *C1QA* (reporter ID: 218232_at), *C1QB* (202953_at), *C2* (203052_at), *C3* (217767_at), *C3AR1* (209906_at), *C5* (205500_at), *C5AR1* (220088_at), *CFB* (202357_s_at), *CFD* (205382_s_at), *CFH* (213800_at), *CFP* (206380_s_at), *CR1* (206244_at) and *CR2* (205544_s_at) were extracted. Candidate genes for either glomerular or tubulointerstital mRNA expression with a correlation threshold of ≥0.5 were used. To identify coexpressed genes among all complement components, the Multiple List Comparator (http://www.molbiotools.com/listcompare.html) was used for comparisons to generate gene lists separated for glomerular and tubulointerstitial compartments. The final gene lists were used for pathway analysis with reactome (http://reactome.org) with a predefined entities value of p≤0.001 (online supplemental tables 4–7).[12]

Statistical methods

For group comparisons, the Mann-Whitney U test was used to determine differences in medians. Spearman's correlation was performed to assess the correlation between levels of serum creatinine, estimated glomerular

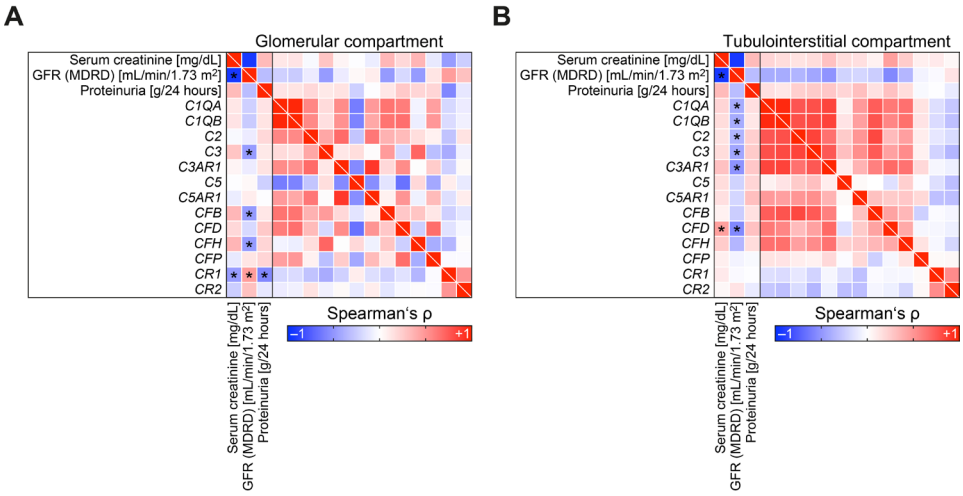

Figure 2 Intrarenal synthesis of distinct complement components associated with impaired kidney function in lupus nephritis. (A,B) Correlations between kidney function parameters and mRNA expression levels of indicated complement components separated for the glomerular and tubulointerstitial compartment in lupus nephritis are shown by heatmap reflecting mean values of Spearman's ρ, asterisks indicate significant associations. GFR, glomerular filtration rate; MDRD, modification of diet in renal disease.

filtration rate (GFR) according to modification of diet in renal disease, proteinuria and median-centred $\log_2$ mRNA expression levels. Heatmaps reflecting the mean values of Spearman's ρ are shown, the asterisks indicating statistical significance correlations. Data analyses were performed with GraphPad Prism (V.9.3.1 for macOS, GraphPad Software, San Diego, California, USA).

RESULTS

From transcriptome datasets, we first extracted mRNA expression levels of complement components *C1QA*, *C1QB*, *C2*, *C3*, *C3AR1*, *C5*, *C5AR1*, *CFB*, *CFD*, *CFH*, *CFP*, *CR1*, and *CR2* specifically from microdissected glomerular (14 healthy controls, 32 with lupus nephritis) and tubulointerstitial compartments (15 healthy controls, 32 with lupus nephritis, (online supplemental tables 1,2).[8] As compared with healthy controls, we observed a significant induction of all complement components exept of *C5*, *CR1* and *CR2* mRNA expression levels in glomerular compartments of lupus nephritis (figure 1A). Accelerated intrarenal synthesis of complement components *C1QB*, *C2*, *C3*, *C3AR1*, *CFB* and *CFD* was independently confirmed in microdissected glomerular compartments of lupus nephritis (6 normal kidneys, 25 with lupus

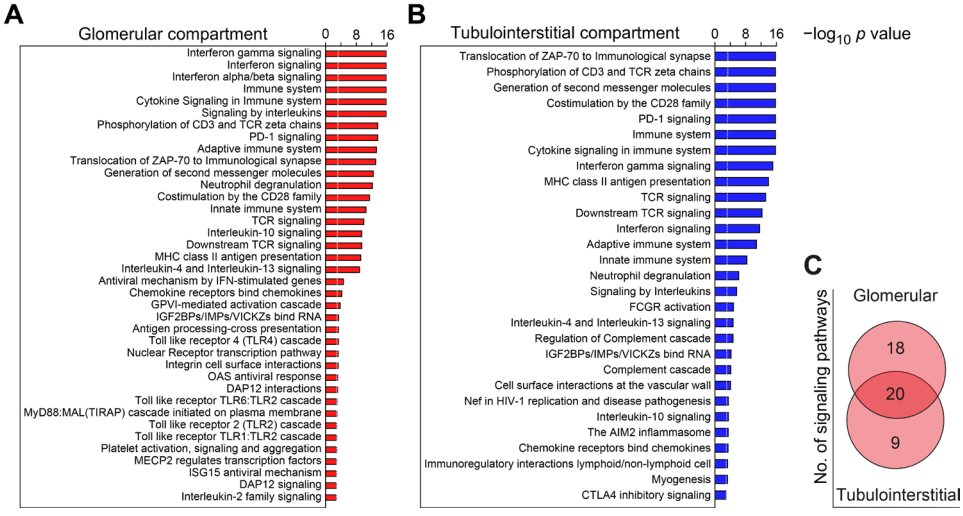

Figure 3 Dissecting signalling pathways associated with intrarenal synthesis of complement components in lupus nephritis. (A,B) Entities $-\log_{10}$ p values of signalling pathways separated for gene set enrichment associated with either glomerular or tubulointerstitial mRNA expression of complement components are shown (the dotted lines correspond to the predefined threshold value of p≤0.001). Glomerular complement synthesis showed the strongest association with interferon signalling and signalling by interleukins, tubulointerstitial complement synthesis with T-cell receptor signalling. (C) Signalling pathways associated with mRNA expression of complement components within the glomerular, tubulointerstitial or both compartments in lupus nephritis are shown.

nephritis, (online supplemental figure 1A) and online supplemental table 3).[9] In the tubulointerstitial compartment, a significant induction of *C1QA*, *C1QB*, *C2*, *C3*, *C3AR1*, *CFB*, *CFD*, *CFH* mRNA expression levels was observed in lupus nephritis as compared with healthy controls (figure 1B). Interestingly, no such induction of intrarenal complement synthesis was observed in disease controls including hypertensive nephropathy, diabetic kidney disease or minimal change disease (online supplemental figure 2A,B).[10 11] As assessed by GFR, glomerular synthesis of complement components *C3*, *CFB* and *CFH* correlated with impaired kidney function in lupus nephritis (figure 2A). Contrasting to this, specifically glomerular *CR1* synthesis was associated with better kidney function and less proteinuria (figure 2A). In the tubulointerstitial compartment, complement components *C1QA*, *C1QB*, *C2*, *C3*, *C3AR1* and *CFD* correlated with GFR loss independent of proteinuria in lupus nephritis (figure 2B). To identify signalling pathways associated with intrarenal complement synthesis in lupus nephritis, we finally performed gene set enrichment identifying 476 common genes that were all associated with glomerular mRNA expression levels of complement components *C1QA*, *C1QB*, *C2*, *C3AR1*, *C5AR1*, *CFD* and *CFP* (online supplemental table 4). Signalling pathway analysis revealed the strongest enrichment of interferon signalling and signalling by interleukins associated with glomerular complement synthesis in lupus nephritis (figure 3A and online supplemental table 5). In the tubulointerstitial compartment of lupus nephritis, gene set enrichment identified 328 common genes that were all associated with mRNA expression levels of complement components *C1QA*, *C1QB* and *C3AR1* (online supplemental table 6). These genes were most associated with T-cell receptor signalling including translocation of ZAP-70 to immunological synapse, phosphorylation of CD3 and TCR zeta chains, generation of second messenger molecules, costimulation by the CD28 family, PD-1 signalling, and cytokine signalling in immune system (figure 3B and online supplemental table 7). Most signalling pathways were enriched in both compartments, while there was also a subset of signalling pathways specific for the glomerular or tubulointerstitial compartment in lupus nephritis (figure 3C).

DISCUSSION

It is long known that complement components are produced by the liver, kidneys, brain, blood vessels and other organs.[13] The in situ deposition of immune complexes from the circulatory system or kidney may promote the accumulation of inflammatory cells and cause kidney damage.[4] Because protein-based detection methods of intrarenal deposits cannot dissect between these sources of complement components, we here specifically analysed transcriptome datasets to systematically describe intrarenal synthesis of complement components in lupus nephritis. We identified accelerated intrarenal synthesis of distinct classical and alternative complement pathway components, most associated with impaired kidney function. Contrasting to this, no such induction of intrarenal complement synthesis was observed in disease controls further supporting relevance of intrarenal complement synthesis especially in human lupus nephritis. Interestingly, specifically glomerular *CR1* synthesis was associated with better kidney function and less proteinuria in lupus nephritis. This is in line with previous studies reporting that loss of CR1 expression correlated with susceptibility to develop SLE and lupus nephritis.[14] Furthermore, we here identified interferon signalling and signalling by interleukins to associate specifically with glomerular complement synthesis in human lupus nephritis. This is in line with observations in animal models reporting that interferon gamma signalling was required for development of experimental lupus nephritis.[15] Finally, we here report that aberrant T-cell receptor signalling predominantly associated with tubulointerstitial complement synthesis. Abnormalities of various molecules in T-cell receptor signalling, including *ZAP70*, have been shown to result in the development of systemic autoimmune diseases including lupus nephritis.[16] The role of the complement system in the pathogenesis of lupus nephritis has long been described, whereas its paradoxical effects on disease activity make it a challenging therapeutic target. Ongoing trials are testing efficacy and safety of anti-C5 antibody (NCT04564339) and C5a receptor (*C5aR*) antagonists (NCT02151409) in patients with lupus nephritis. This study provides additional insights into signalling pathways associated with intrarenal synthesis of complement components in lupus nephritis that might be also affected by targeted therapy of the complement system.

Correction notice This article has been corrected since it was first published online. Reference 15 has been added to the discussion section.

Contributors BT conceived the letter, analysed data and wrote the manuscript. DT performed gene set enrichment analysis. SH edited the manuscript. All authors reviewed and approved the manuscript's content before submission.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. Data were extracted from Nephroseq Research Edition (Ann Arbor, Michigan: University of Michigan; available from: www.nephroseq.org). Participants gave informed consent to participate in the study before taking part.

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