Supplemental Methods

C5 inhibition prevents renal failure in a mouse model of lethal C3 glomerulopathy

Allison Lesher Williams[1], Damodar Gullipalli[1], Yoshiyasu Ueda[1], Sayaka Sato[1], Lin Zhou[1], Takashi Miwa[1], Kenneth S. Tung[2] and Wen-Chao Song[1,*]

ELISAs

For intact C5, plates were coated with 5μg/ml anti-mouse C5 mAb BB5.1 in 0.1M carbonate buffer overnight at 4°C. Plates were then blocked with 1% BSA-PBS for 1h at RT, followed by addition of serially diluted mouse plasma samples, starting at 1:10 in 1%BSA-PBS. Positive control was WT plasma (same plasma used for all plates). After incubation with plasma for 1h at RT, plates were washed 3 times with PBS-T and then incubated with a goat polyclonal anti-human C5 Ab (cross-reacts with mouse C5, 1:1000, Quidel, CA) for 1h at RT. Finally, plates were incubated with HRP rabbit anti-goat IgG Ab (1:4000, Bio-Rad) for 1h at RT. After washing, plates were developed using OptEIA substrate (BD Biosciences). All samples were normalized to the positive control (WT plasma). Intact and activated C3 levels were measured as described previously.[1]

C5 Western blotting

Mouse plasma (1μl) was diluted with sample buffer and boiled before loading onto 8% SDS-PAGE gels under non-reducing conditions. Samples were then transferred to PVDF membrane. Blots were probed first with goat anti-human C5 Ab (cross-reacts with mouse C5; 1:1000, Quidel) followed by detection with HRP goat-anti rabbit IgG (1:4000, Bio-Rad).

fH depletion from mouse serum

fH depletion from mouse serum and subsequent serum processing was performed as described previously.[16] Intact C5 levels remaining in serum were measured by ELISA as described above.

Survival curve

Survival was assessed in treatment groups over respective time periods noted for each condition. Data were categorized as being censored (euthanized) or natural death and analyzed by GraphPad Prism (La Jolla, CA) using the Mantel-Haenszel log rank test as described previously.[2]

Histology

For light microscopy, kidneys were fixed in 10% formalin-PBS before processing and paraffin embedding. Samples were cut in 4µm sections and stained with H&E or periodic acid Schiff (PAS) reagent. Kidneys were snap-frozen in optimal cutting temperature (OCT) medium and stored at -80°C. For immunofluorescence studies, 4µm sections were cut and used for staining. For C3, FITC-conjugated goat anti-mouse C3 Ab was used (1:500, MP Biomedicals). C9 was visualized by staining with rabbit anti-rat C9 (1:500, from Dr. Paul Morgan, Cardiff University) followed by staining with FITC-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology). For detecting CD11b-positive cells, a FITC-conjugated rat anti-mouse CD11b IgG was used with a FITC rat IgG2b used as an isotype control (1:50 for both antibodies, BD Pharmingen). CD11b-positive cells were counted in 20-30 glomeruli and averaged for each mouse sample. For interstitial tissue, 10 separate fields at 400x magnification were counted from different sections of tissue and then averaged for each sample.

Electron microscopy

Electron microscopic examination of kidneys was performed as described previously.[1]

Quantification of immunofluorescence staining (C3, C9)

After staining for the protein of interest (C3, C9), glomerular fluorescence staining was analyzed with ImageJ software using a protocol adapted from previously published work.[3] Briefly, images were first converted to grayscale. Then for each glomerulus, a drawing tool was used to select the area of glomerulus to quantify area, integrated density and mean gray value. A small area of background next to the glomerulus was also measured using the same parameters. This process was repeated in 20-30 glomeruli for each sample. The mean gray values for the background measurements were averaged to serve as a single background value. This value was then used to calculate the corrected total cell fluorescence (CTCF) as follows: CTCF = integrated density – (glomerular area x mean background fluorescence). CTCF values of glomeruli were then divided by total area to normalize CTCF values per unit. These values were then averaged for each mouse sample for subsequent analysis.

Quantification of CD11b+ cells

After staining as described, CD11b-positive cells were counted in 20-30 glomeruli and averaged for each mouse sample. For interstitial tissue, 10 separate fields at 400x magnification were counted from different sections of tissue and then averaged for each sample.

Statistical analysis

Data were analyzed with GraphPad software by 2-tailed Student's t test or one-way ANOVA with Tukey's test for parametric data. Normality of data was assessed by the Kolmogorov-Smirnov test. A p value less than 0.05 was considered statistically significant. Error bars indicate standard error of the mean (SEM) unless indicated otherwise.

References:

1. Lesher AM, Zhou L, Kimura Y*, et al.* Combination of factor H mutation and properdin deficiency causes severe C3 glomerulopathy. *J Am Soc Nephrol* 2013; **24:** 53-65.

2. Altman DG. *Practical Statistics for Medical Research*, 1st ed. edn. Chapman & Hall: Oxford, UK, 1991.

3. Burgess A, Vigneron S, Brioudes E*, et al.* Loss of human Greatwall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. *PNAS* 2010; **107:** 12564-12569.