Prevention of High Fat Diet-Induced Podocyte Injury and Glomerular Sclerosis in Mice Lacking Nod-Like Receptor Protein 3: Role of Inflammasome Extinction

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ABSTRACT

Recent studies have demonstrated that NLRP3 inflammasome formation and activation are an important early mechanism responsible for podocyte injury and glomerular inflammation in obese mice. However, it remains unknown whether Nlrp3 gene is critical for the activation of inflammasomes in glomeruli of obese mice. To answer this question, the Nlrp3 knockout (*Nlrp3*$^{-/-}$) and wild type (*Nlrp3*$^{+/+}$) mice were fed a high fat diet (HFD) or normal chow (ND) for 12 weeks to produce obesity. Confocal microscopic analysis showed that HFD increased the colocalization of NLRP3 with Asc or caspase-1 in glomeruli of *Nlrp3*$^{+/+}$, but not in *Nlrp3*$^{-/-}$ mice, suggesting that obesity-enhanced formation of Nlrp3 inflammasomes. Furthermore, colocalization of Nlrp3 with podocin indicates the enrichment of Nlrp3 in podocytes. In addition, biochemical analysis demonstrated that HFD significantly increased the caspase-1 activity and IL-1β production in *Nlrp3*$^{+/+}$ mice, but not in *Nlrp3*$^{-/-}$ mice. Correspondingly, the urinary protein and albumin excretion were significantly higher in *Nalp3*$^{+/+}$ mice compared to ND fed mice. However, the HFD-induced increase in urinary protein and albumin were significantly lowered in *Nlrp3*$^{-/-}$ compared to *Nlrp3*$^{+/+}$ mice. In addition, Western blot analysis showed that HFD significantly increased the desmin, HMGB-1 and RAGE expression in glomeruli of *Nlrp3*$^{+/+}$ mice, but not in *Nlrp3*$^{-/-}$ mice. Based on these results, it is concluded that Nlrp3 is an essential component of Nlrp3 inflammasomes and that extinction of Nlrp3 inflammasomes in podocytes protects podocytes and glomeruli from obesity-induced injury (supported by NIH grants DK54927, HL-091464 and HL-75316).

METHODS

Animals: Eight week old male Nlrp3 knockout (Nlrp3$^{-/-}$) and wild type (Nlrp3$^{+/+}$) mice were randomized to receive normal diet or High fat diet (HFD) (Research Diets Inc., Bethlehem, PA) for 12 weeks .

Morphological examinations. The fixed kidneys were paraffin-embedded, sections were prepared and stained with periodic acid–Schiff stain (3). Glomerular injury index was calculated from 0 to 4 on the basis of the degree of glomerulosclerosis and mesangial matrix expansion as described previously. In general, we counted a total of 80-100 glomeruli in each kidney slice under the microscope, where each glomerulus was graded 0-4 damages. 0 represents no lesion, 1+ represents sclerosis of <25% of the glomerulus, while 2+, 3+, and 4+ represent sclerosis of 25% to 50%, >50% to 75%, and >75% of the glomerulus. A whole kidney average sclerosis index was obtained by averaging scores from counted glomeruli.

Biochemical Analysis, urinary total protein and albumin excretion measurement: The 24-hour urine samples were collected using metabolic cages and subjected to urinary albumin excretion measurements. Urine albumin was detected by using a commercially available mouse albumin enzyme-linked immunoassay assay kit (Bethyl Laboratories, Montgomery, TX, USA) as we described previously (2). Caspase activity was determined using a commercially available kit (Biovision, Mountain View, CA, USA). IL-1β concentration was determined using the mouse ELISA kit (R & D systems, Minneapolis, MN, USA).

Double immunoflourescent staining and confocal microscopy: Double immunoflourescent analysis of renal tissue was performed to detect co-localization of Nlrp3 with Asc and Nlrp3 with caspasae-1 (Abcam, Cambridge, MA, USA , 1: 200 dilution) antibodies (4).

Western blot analysis: Homogenates were prepared from the cortex tissue with a modified method as we described previously (4). Desmin, HMGB1 and RAGE protein expressions in the kidney from Nlrp3$^{+/+}$ and Nlrp3$^{-/-}$ mice were detected by Western blot analysis with a monoclonal antibody against Desmin (1:500 dilution for overnight at 4°C, BD Biosciences, San Diego, CA, USA), HMGB1 and RAGE(1:500 dilution for overnight at 4°C, Abcam, Cambridge, MA, USA). For normalization, the blots were reprobed with alternative primary antibody against the housekeeping protein β-actin (1:2000 dilution for 1 hour, Sigma).

BACKGROUND

Obesity is one of the leading health problems in the United States. Its prevalence increased dramatically over the last 20 years. Clinical and epidemiological studies suggest that obesity may cause glomerular injury leading to end-stage renal disease (ESRD) in addition to hypertension and diabetes.

A few mechanisms have been proposed for the obesity-induced chronic kidney disease, including chronic inflammation, abnormal vascular remodeling, rise in renal plasma flow, hyperfiltration, and renal lipotoxicity. However, the mechanisms of obesity-induced renal injury, especially the early changes to initiate diabetic nephropathy are still poorly understood.

Inflammasome is a multiprotein complex responsible for the activation of caspase-1, which cleaves IL-1β and switches on the inflammatory process when cells are challenged by some endogenous or exogenous danger signals (1).

NLRP3 inflammasome has been implicated in the pathogenesis of various metabolic diseases including diabetes, obesity, gout, silicosis, liver toxicity and acute myocardial infarction (1).

Recently it has been shown that obesity leads to NLRP3 inflammasome formation and activation in podocytes, leading to glomerular dysfunction and sclerosis (2). However, it remains unknown whether Nlrp3 gene is critical for the activation of inflammasomes in glomeruli of obese mice.

RESULTS

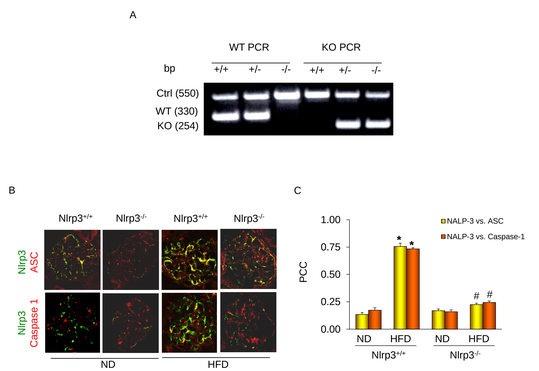

Fig. 1: Genotyping in Nlrp3 mice. Two PCR products suggest heterozygous mutation, while single band represents wild type or knockout allele (Panel A). Confocal microscopy showed that colocalization of Nlrp3 *vs.* ASC or caspase-1 was increased as shown by yellow spots in glomeruli (Panel B), suggesting enhanced formation of Nlrp3 inflammasomes in glomeruli of Nlrp3$^{+/+}$ mice fed a HFD. In Nlrp3$^{-/-}$ mice, however, such colocalizations were not detected. The Pearson correlation coefficient (PCC) was calculated for each of the groups and summarized in panel C. * significant difference from normal diet fed mice; # Significant difference from Nlrp3$^{+/+}$ mice fed a HFD.

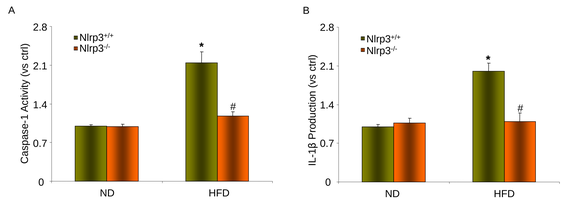

Fig. 2: Biochemical analysis demonstrated that high fat diet significantly increased the caspase-1 activity and IL-1β production in glomeruli of Nlrp3$^{+/+}$ mice (Panel A and B). However, HFD-induced activation of Nlrp3 inflammasomes was not observed in Nlrp3$^{-/-}$ mice, suggesting the essential role of Nlrp3 in inflammasome activation. ND: Normal diet, HFD: high fat diet. * significant difference from ND fed mice; # significant difference from Nlrp3$^{+/+}$ mice fed a HFD.

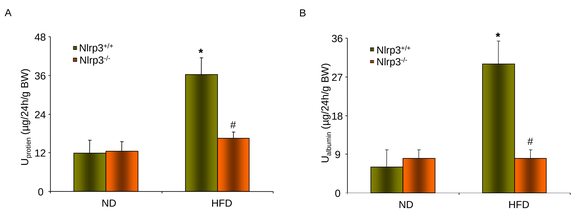

Fig. 3: Attenuation of obesity-induced glomerular injury in mice lacking Nlrp3 gene. The 24h urinary protein excretion (Panel A) and albumin excretion (Panel B) were increased significantly in HFD fed Nlrp3$^{+/+}$ mice. However, the HFD-induced urinary protein and albumin excretion were attenuated in Nlrp3$^{-/-}$ mice. These results suggest that Nlrp3 gene inhibition protects obesity-induced glomerular injury in mice**.** ND: Normal Diet; HFD: High fat diet. * significant difference from ND fed mice; # significant difference from Nlrp3$^{+/+}$ mice fed a HFD.

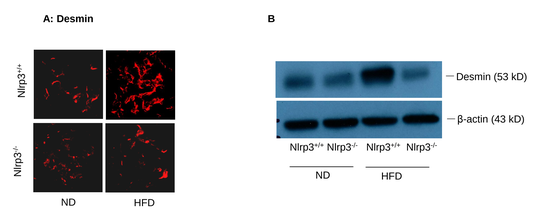

Fig. 4: Immunohistochemical analysis revealed that podocyte injury was ameliorated in Nlrp3 knockout mice. Typical images of desmin staining in glomeruli are from 4 groups of mice, and HFD increased the desmin staining in Nlrp3$^{+/+}$ mice than in Nlrp3$^{-/-}$ mice (Panel A). In addition, Western blot analysis further confirmed that HFD increased the desmin expression in Nlrp3$^{+/+}$ compared to normal diet fed mice, but not in Nlrp3$^{-/-}$ mice. These data revealed that obesity-induced podocyte injury was attenuated in mice lacking Nlrp3 gene (Panel B). ND: Normal diet; HFD: High fat diet.

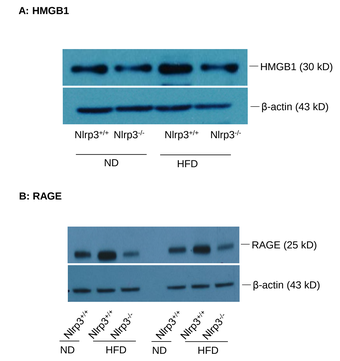

Fig. 5: Using antibodies against high mobility group box (HMGB1), a prototype of danger associated molecular pattern and receptor for advanced glycation end products (RAGE), Western blot analysis showed that high fat diet treatment increased HMBG1 and RAGE in the renal cortex tissue of Nlrp3$^{+/+}$mice. However, the Nlrp3$^{-/-}$ mice had significantly less HMBG1 and RAGE expression when compared to Nlrp3$^{+/+}$ mice on the high fat diet. These results suggest that activation of intracellular inflammasomes has both inflammatory and non-inflammatory action in glomeruli during obesity, which ultimately initiate and promote the development of glomerular injury/sclerosis.

CONCLUSION

It is concluded that Nlrp3 is an essential component of Nlrp3 inflammasomes and that targeting Nlrp3 may be important therapeutic strategy to prevent inflammasome formation and thereby protect podocytes from obesity-induced injury.

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