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 Nlrp3−/− mice. To answer this question, the Nlrp3 knock-out (Nlrp3−/−) and wild type (Nlrp3+/+) mice were fed a high fat diet (HFD) or normal chow (ND) for 12 weeks to produce obesity. Cytological 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-induced formation of NLRP3 inflammasomes. Furthermore, colocalization of Nlrp3 with podocyte indicates the enrichment of Nlrp3 in podocytes. In addition, biochemical analysis demonstrated that HFD significantly increased the expression of IL-1β production in Nlrp3−/− mice, but not in Nlrp3+/+ mice. Correspondingly, the urinary protein and albumin excretion were significantly higher in Nlrp3−/− 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 density, HMBG1 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 activation of Nlrp3 inflammasomes in podocytes precedes glomeruli and obesity-induced injury (supported by NIH grants DK54457, R01-HL01464 and R01-HL75355).

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

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

RESULTS

Fig. 1: Glucosinylating in Nlrp3 mice. Two PCE probes suggest heterogeneous mutation, which single band represents (Type of knock-out allele (Panel A)). Cytological microscopic analysis showed that colocalization of Nlrp3 vs. ASC or caspase-1 was increased as showed by yellow or green color dot (Panel B). These findings revealed that obesity-induced inflammasomes in glomeruli of Nlrp3−/− mice fed a HFD. In Nlrp3−/− mice, such colocalization was 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 ND fed mice (Panel B). ND: Normal diet; HFD: High fat diet.

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: Immunohistological analysis revealed that podocyte injury was augmented in Nlrp3 knock-out mice. Typical images of glomeruli in glomeruli fed from 4 groups of mice and HFD increased the density staining in Nlrp3−/− mice than in Nlrp3+/+ mice (Panel C). In addition, Western blot analysis further confirmed that HFD increased the density expression in Nlrp3−/− mice as compared to normal diet fed mice. But not in Nlrp3+/+ mice. These data revealed that obesity-induced podocyte injury was augmented in mice lacking Nlrp3 gene (Panel B). ND: Normal diet; HFD: High fat diet.

Fig. 4: Immunohistological analysis revealed that podocyte injury was augmented in Nlrp3 knock-out mice. Typical images of glomeruli in glomeruli fed from 4 groups of mice and HFD increased the density staining in Nlrp3−/− mice than in Nlrp3+/+ mice (Panel C). In addition, Western blot analysis further confirmed that HFD increased the density expression in Nlrp3−/− mice as compared to normal diet fed mice. But not in Nlrp3+/+ mice. These data revealed that obesity-induced podocyte injury was augmented in mice lacking Nlrp3 gene (Panel B). ND: Normal diet; HFD: High fat diet.

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