Mincle maintains M1 macrophage activation via TLR4/NF-κB to promote renal inflammation

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INTRODUCTION

Mincle is a transmembrane pattern recognition receptor (PRR) involving the innate immunity, but its role in kidney disease is still unexplored.

We report here the pathogenic importance of Mincle in M1 macrophage-mediated acute renal inflammation.

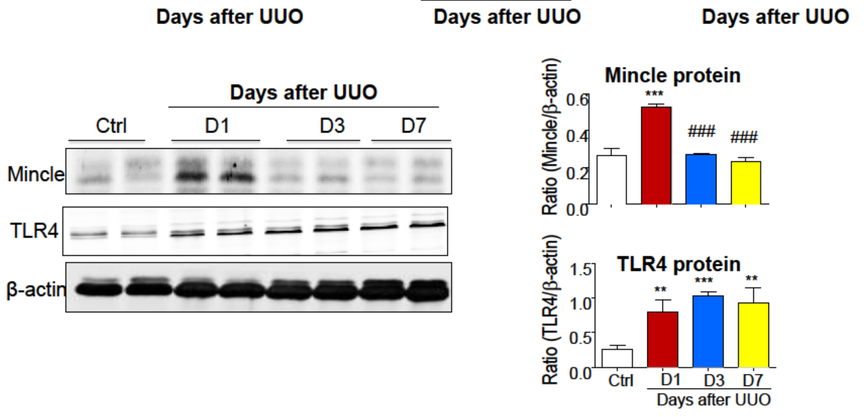
METHOD

Unilateral ureteral obstructive (UUO)-induced chronic kidney disease (CKD) and cisplatin-induced acute kidney injury (AKI) mouse models were used in this study. *In vitro* study was performed with RAW264.7 and bone marrow derived macrophage to investigate the underlie mechanism by which Mincle participated in renal inflammation.

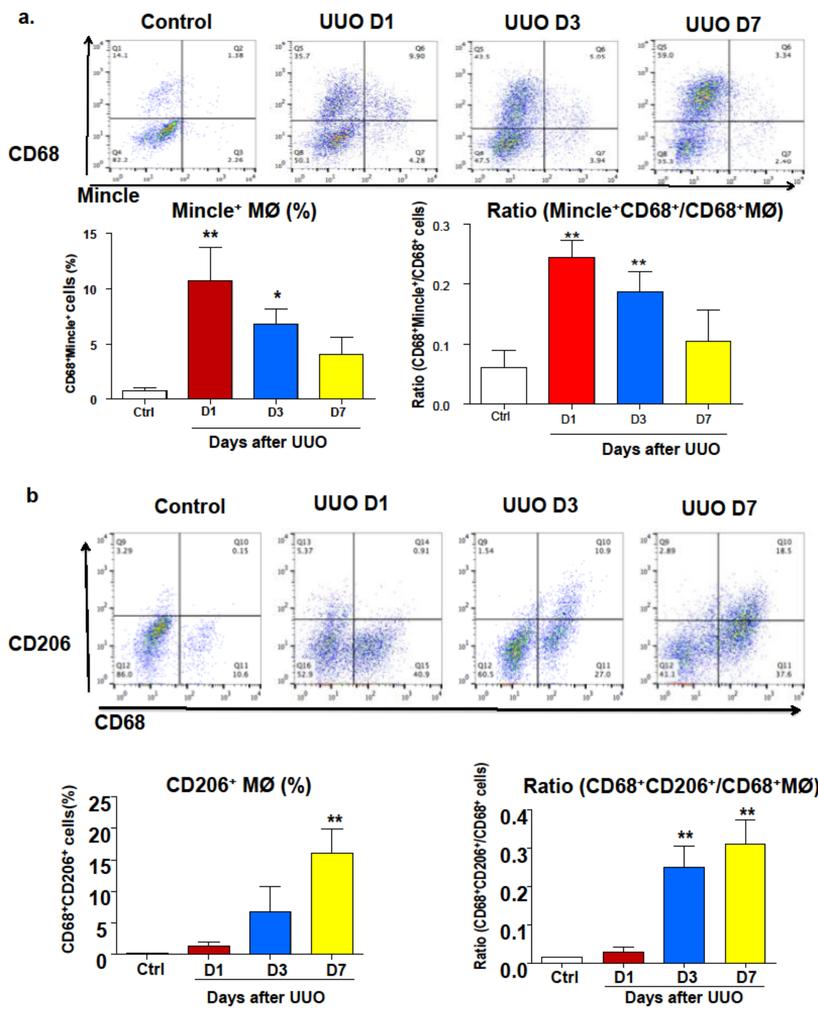
RESULTS

1. Mincle is induced at the early stage of kidney injury in UUO model

(A) Mincle upregulation was associated with macrophage infiltration and TLR4 expression

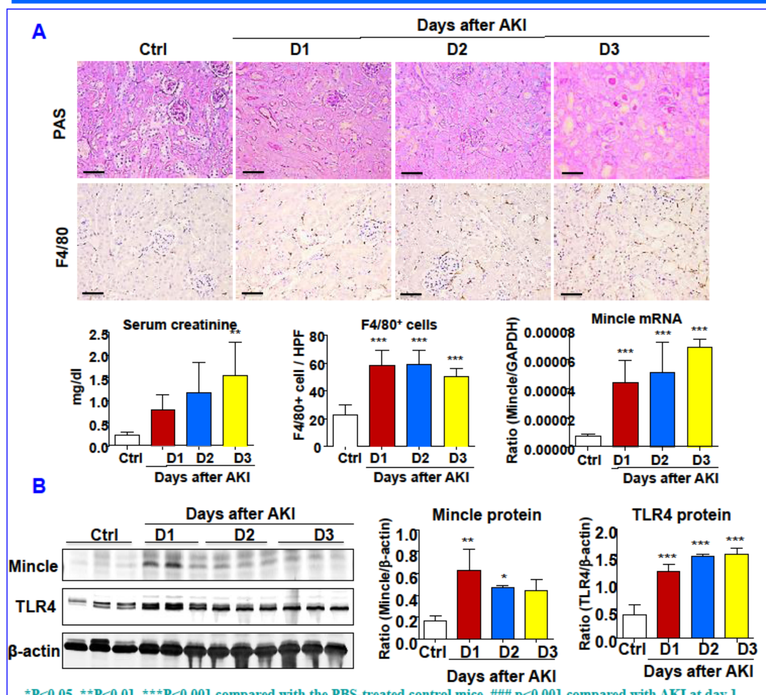


(B)Two-color flow cytometry detects Mincle-expressing macrophages which was correlated with M1 macrophage in the early stage of the UUO kidney.



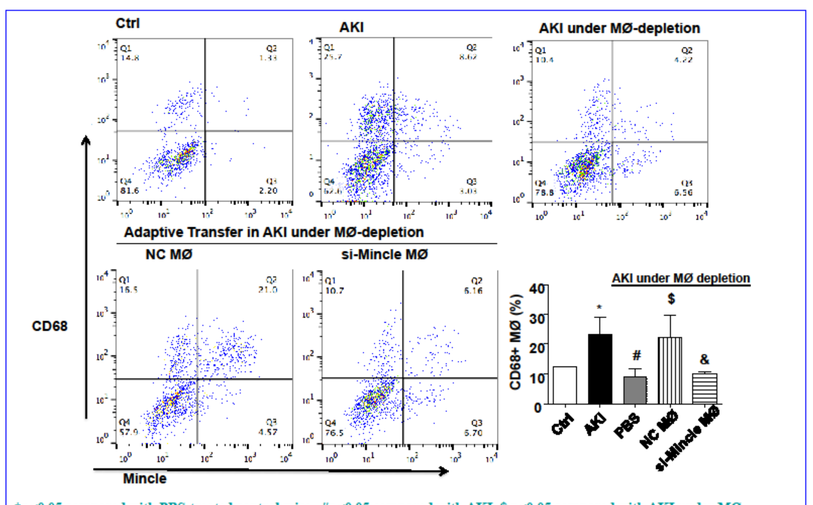
*P<0.05, **P<0.01, ***P<0.001 compared with the control sham mice. ###P<0.001, as compared with day 1 (D1) UUO kidney.

2. De novo expression of Mincle at the early stage of cisplatin-induced AKI.



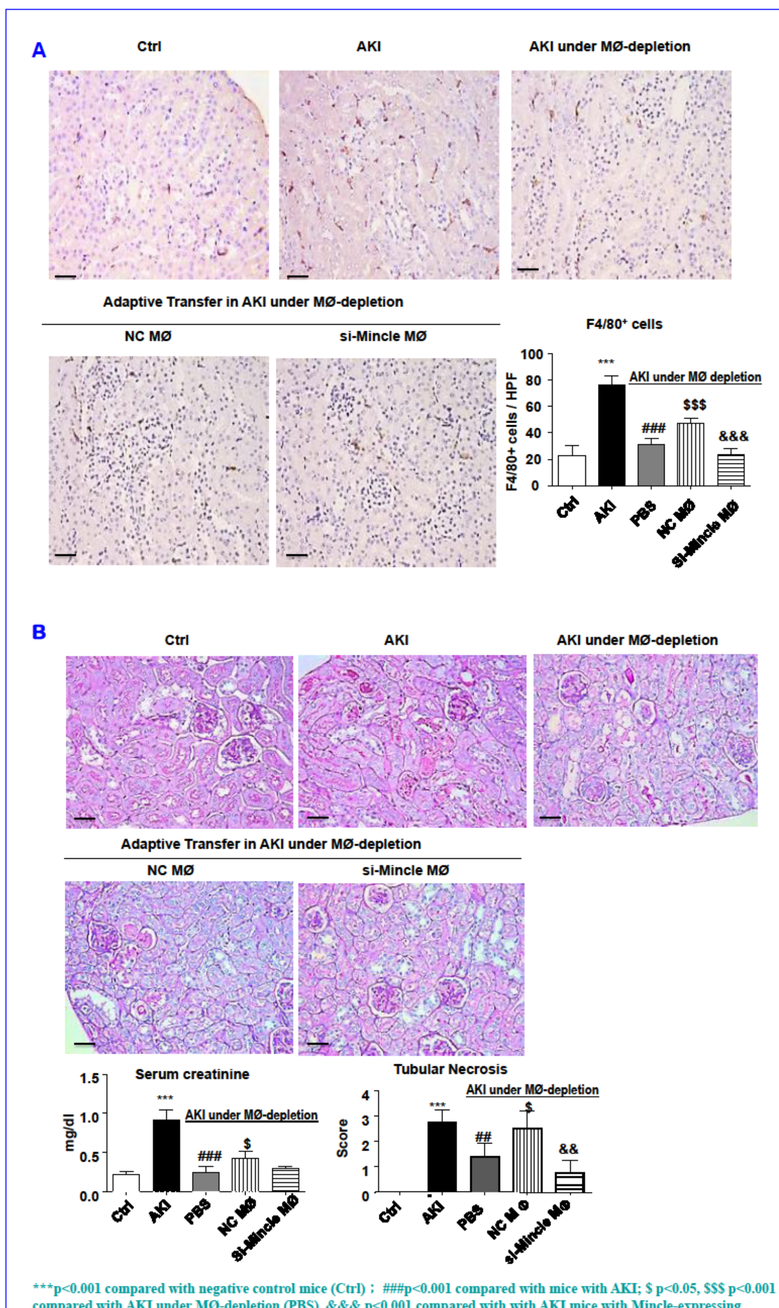
*P<0.05, **P<0.01, ***P<0.001 compared with the PBS-treated control mice. ### p<0.001 compared with AKI at day 1. Magnifications: x400.

3. Flow cytometry analysis of CD68+Mincle+ macrophages in the day 1 AKI kidney with or without adoptive macrophage transfer in macrophage depletion LysM-Cre/DTR mice.



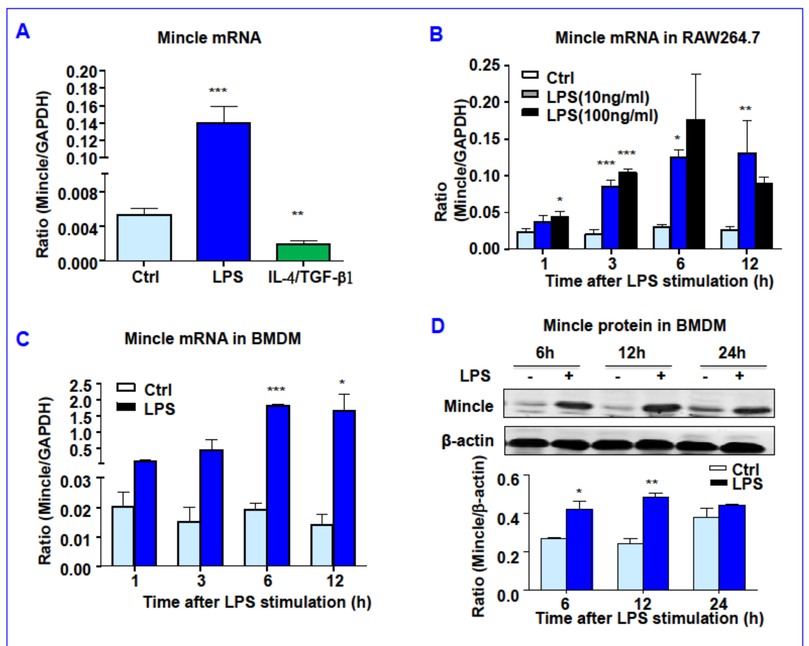
*p<0.05 compared with PBS-treated control mice, #p<0.05 compared with AKI, $ p<0.05 compared with AKI under MØ-depletion, & p<0.05 compared with AKI mice with Mincle-expressing macrophage transfer (NC Mø).

4. Adoptive transfer of Mincle-knockdown macrophages reduces kidney injury in cisplatin-induced AKI mice at day 3.



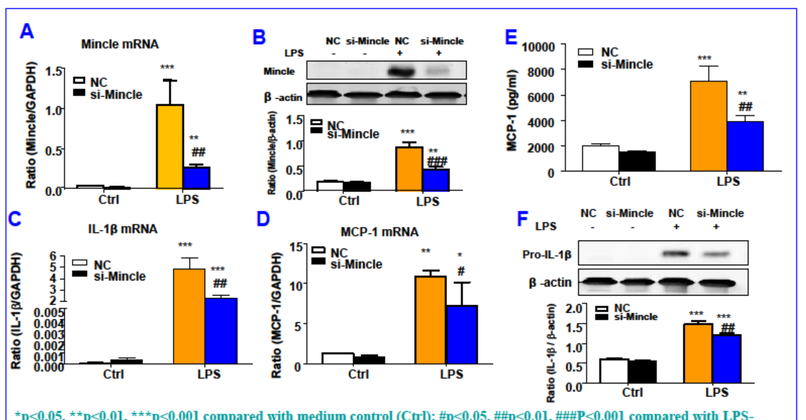
***p<0.001 compared with negative control mice (Ctrl) ; ###p<0.001 compared with mice with AKI; $ p<0.05, $$$ p<0.001 compared with AKI under MØ-depletion (PBS), &&& p<0.001 compared with with AKI mice with Mincle-expressing macrophage transfer (NC Mø). Magnifications: x400.

5. Mincle is induced by LPS and expressed by M1 macrophages in but not by M2 macrophages in vitro.



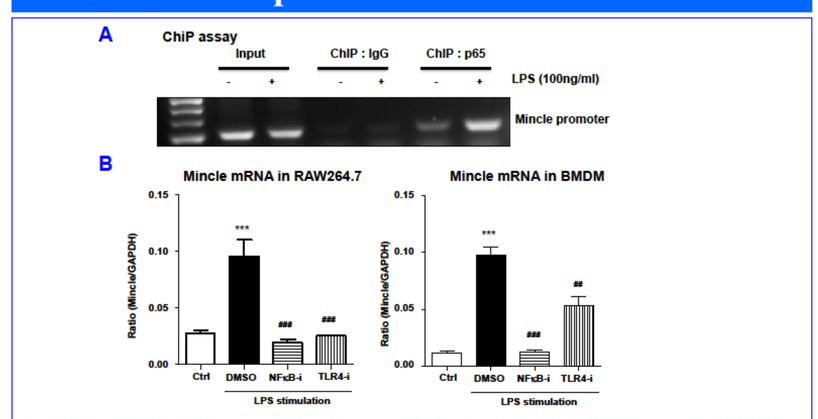
*p <0.05, **p <0.01, ***p <0.001 compared with untreated controls.

6. Mincle is essential for maintaining M1 macrophage phenotype



*p<0.05, **p<0.01, ***p<0.001 compared with medium control (Ctrl); #p<0.05, ##p<0.01, ###P<0.001 compared with LPS-stimulated macrophages without si-Mincle (NC).

7. Mincle expression by M1 macrophages via a TLR4/NF-κb-dependent mechanism



*p<0.05, **p<0.01, ***p<0.001 compared with medium control (Ctrl) or time 0; ##p<0.01, ###P<0.001 compared with DMSO control. BMDM, bone marrow derived macrophage.

CONCLUSION

Our data revealed that Mincle was specifically expressed by M1 macrophages and was a novel promoter triggering acute renal inflammation. Mincle was tightly regulated by the TLR4/NF-kB pathway to maintain the M1 macrophage phenotype. Importantly, we also demonstrated that targeting Mincle on macrophages was able to inhibit M1 macrophage-mediated acute kidney injury.

ACKNOWLEDGEMENT

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