No. 2131

Elucidation of Cytokine Secretion Regulatory Mechanism from Macrophages by Salt Loading through WNK Signal

Eisei Sohara

Tokyo Medical and Dental University, Department of Nephrology

Summary

With-no-lysine kinase (WNK) plays important roles in regulating electrolyte homeostasis, cell signaling, survival, and proliferation. It has been recently demonstrated that WNK1, a member of the WNK family, modifies the function of immune cells. Here we report that in macrophages, WNK1 has suppressive effects on lipopolysaccharide (LPS)-induced inflammatory responses via TGFβ-activated kinase 1 (TAK1)-mediated activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathway.

We investigated the effect of lipopolysaccharide (LPS; 10 ng/ml) stimulation on WNK1 expression in RAW 264.7 cells, a murine macrophage cell line, and peritoneal macrophages (pMΦ). Then, we evaluated the effect of WNK1 silencing on LPS-induced cytokine production using small hairpin RNA (shRNA) and WNK1+/- mice.

We found that WNK1 heterozygous (WNK1+/-) mice produced excessive proinflammatory cytokines in an experimental LPS-induced sepsis model, and peritoneal macrophages isolated from WNK1+/- mice produced higher levels of LPS-induced cytokines and NOS2 expression as canonical proinflammatory M1 macrophage markers. We confirmed that small hairpin RNA (shRNA)-mediated knockdown of WNK1 activated LPS-induced cytokine production and NOS2 expression in RAW 264.7 macrophages. Moreover, we demonstrated that WNK1 knockdown increased the nuclear translocation of NF-κB and activated the p38 and Jun N-terminal kinase (JNK) MAPK signaling pathway and that a TAK1 inhibitor diminished these effects of WNK1 knockdown.

WNK1 has multiple essential physiological functions in diverse tissues. Mutations in the gene encoding WNK1 were first identified by positional cloning causing familial hyperkalemic hypertension, and a major focus to dissect its functions has been in the context of renal regulation of ion transport. In extrarenal tissues, recent studies have revealed that WNK1 is ubiquitously expressed and regulates fundamental cellular functions, including cell differentiation and development. However, the definite physiological roles of WNK1 have remained largely unknown because homozygous WNK1 deletion is embryonically lethal. This study is the first to clarify that WNK1 suppresses LPS-induced proinflammatory cytokine production and classical activation using pMϕ isolated from WNK1+/− mice. Moreover, we showed that WNK1+/− mice produced excessive proinflammatory cytokines in an experimental LPS-induced sepsis model *in vivo*.

Our study suggests that WNK1 acts as a physiologic immune modulator via interactions with TAK1. WNK1 may be a therapeutic target against the cytokine storm caused by sepsis.