Polo-like kinase 1 protects intestinal epithelial cells from apoptosis during sepsis via the nuclear factor-κB pathway

Ying-Ya Cao[1], Zhen Wang[1], Lin-Ming Lu[2], Zeng-Xiang Xu[2], Jia-Jia Li[2], Xiao-Gan Jiang[1], Wei-Hua Lu[1]

[1]Department of Intensive Care Unit, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui 241001, China;
[2]Department of Pathology, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui 241001, China.

To the Editor: Sepsis is defined as the life-threatening organ dysfunction caused by dysregulated host response to infection, eventually leading to multiple organ dysfunction syndrome. The gut, which is considered to play a significant role during sepsis, is defined as the motor of sepsis. During sepsis, increasing epithelial apoptosis damages gut integrity and weakens cell adherence, eventually leading to intestinal barrier failure and aggravating the degree of sepsis.[1] The nuclear factor (NF)-κB pathway plays an important role in intestinal mucosal barrier failure during sepsis. However, whether NF-κB activation plays a harmful or beneficial role in the intestine in response to inflammation remains a matter of debate.[2] Here, we established a model of lipopolysaccharide (LPS)-induced intestinal sepsis *in vitro* and measured the apoptosis in sepsis intestinal tissues to explore the function of NF-κB pathway in sepsis.

Human colorectal cancer cell line HT-29 were incubated with LPS at various concentrations and vehicle-treated cells were used as controls. Apoptotic cells were double-labeled using the Annexin V/fluoresceine isothiocyanate kit (Neo Bioscience, Beijing, China) and analyzed with a BD™ LSR II flow cytometer (BD Biosciences, NJ, USA). Immunofluorescence was used to detect the location of NF-κB p65. Western blot assays were performed using the following antibodies: anti-human polo-like kinase 1 (PLK1) (Abcam, Cambridge, UK), anti-pro-caspase-3 (Abcam), anti-IκB-α (Abcam), and anti-β-actin (Abcam) antibodies. We also selected 21 intestinal obstruction or perforation participants met the following eligibility criteria: septic shock patients who met the definition of septic shock 3.0 and needed surgery as assessed by the surgeons, and had a pathological diagnosis of intestinal inflammatory cell infiltration. Twenty-one surgically resected necrotic intestinal and operative margin tissues that were morphologically normal were collected. All tissues were formalin-fixed and paraffin-embedded routinely. Then paraffin-embedded tissues were immunohistochemistry stained with following antibodies: anti-PLK1 (Abcam), anti-NF-κB p65 (Abcam), anti-pro-caspase-3 (Abcam), and anti-pro-caspase-9 (Abcam) antibodies, and results were evaluated blindly by two observers. All analyses were performed using the SPSS software program (SPSS Standard version 19.0, Chicago, IL, USA).

NF-κB pathway is a classic signaling pathway in the inflammatory response. Our results found that the expression of IκB-α was markedly reduced with LPS treatment [Figure 1A]; meanwhile, NF-κB p65 protein showed increased nuclear localization with LPS stimulation in HT29 cells [Figure 1B]. Those results showed that LPS treatments activated the NF-κB pathway. To verify the importance of activation of NF-κB in LPS-induced apoptosis, HT29 cells were pre-treated with various concentrations of pyrrolidine dithiocarbamic acid (PDTC) to suppress the activity of NF-κB and then were treated with 30 μg/mL LPS for 24 h. Then we found that pre-treatment with PDTC significantly increased the expression levels of pro-caspase-3 and IκB-α [Figure 1C], which illustrates that inhibition of NF-κB reduced LPS-induced apoptosis in HT29 cells. Our previous study showed that LPS down-regulated PLK1 expression in HT29 cells.[3] Down-regulation of PLK1 and activation of NF-κB both might be important in LPS induced apoptosis in HT29 cells. To define the relationship between NF-κB and PLK1 in sepsis, we exposed HT29 cells to BI2536, an inhibitor of PLK1, and examined NF-κB activity. Cells treated with BI2536 exhibited decreased IκB-α expression [Figure 1D] and increased nuclear localization of NF-κB p65 [Figure 1E]. Next, HT29 cells were pre-treated with PDTC (20 ng/mL) to suppress NF-κB activity and then treated with 30 μg/mL LPS for 24 h, followed by detection of PLK1 expression. Importantly, pre-treatment with PDTC had no effect on the expression of PLK1 [Figure 1F]. These observations revealed that inhibition of PLK1 activates the NF-κB pathway, resulting in the induction of apoptosis in

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000780

Correspondence to: Wei-Hua Lu, Department of Intensive Care Unit, Yijishan Hospital, First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui 241001, China
E-Mail: lwh683@126.com

Chinese Medical Journal 2020;133(15)

Received: 25-02-2020 **Edited by:** Qiang Shi

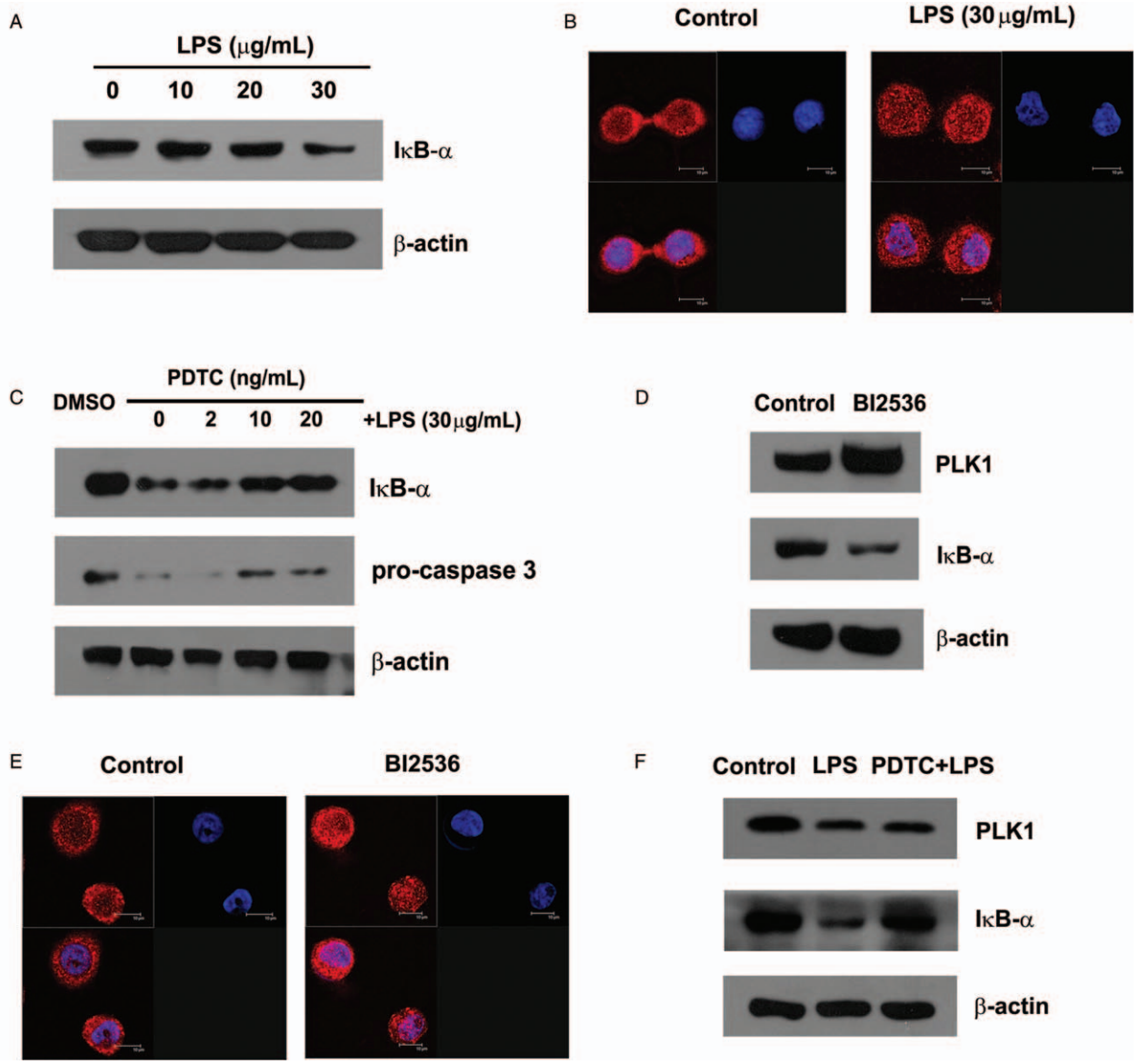

Figure 1: The role of NF-κB in LPS-induced apoptosis in HT29 cells. (A) The expression level of IκB-α in HT29 cells after treatment with different concentrations of LPS for 24 h. (B) NF-κB translocated to the nucleus after treatment with 30 μg/mL LPS for 24 h. Immunofluorescence staining. Red, NF-κB; blue, nuclei. Original magnification ×400. (C) The expression level of pro-caspase-3 and IκB-α in HT29 cells after treatment with PDTC and LPS. (D) The expression levels of PLK1 and IκB-α in HT29 cells after treatment with BI2536 (50 nmol/L) for 24 h. (E) NF-κB was translocated to the nucleus after treatment with BI2536 (50 nmol/L) for 24 h. Immunofluorescence staining. Red, NF-κB; blue, nuclei. Original magnification ×400. (F) The expression level of PLK1 and IκB-α in HT29 cells after treatment with PDTC (20 ng/mL) and LPS (30 μg/mL). DMSO: Dimethyl sulfoxide; LPS: Lipopolysaccharide; NF-κB: Nuclear factor-κB; PDTC: Pyrrolidine dithiocarbamic acid; PLK1: Polo-like kinase 1.

HT29 cells. We also selected clinical-pathological specimens to validate our findings. The results showed the expression levels of pro-caspase-3 and pro-caspase-9 were higher while the expression of PLK1 was lower in adjacent normal operative intestinal tissues than in the necrotic intestinal tissues [Supplementary Figure 1A and 1B, http://links.lww.com/CM9/A214]. Nuclear expression of NF-κB p65, which is indicative of activation of NF-κB signaling, was not found in adjacent normal operative intestinal tissues but was found in intestinal epithelial cells in necrotic intestinal tissues [Supplementary Figure 1A, http://links.lww.com/CM9/A214].

Together with our previous study, this study revealed that inhibition of NF-κB protects gut epithelial cells from apoptosis during sepsis, and this pathophysiology may be regulated by PLK1. Intestinal barrier failure plays a critical role in sepsis and the integrity of the intestinal barrier relies on the balance of epithelial cell proliferation and apoptosis. Our results revealed decreased expression of pro-caspase-3, a marker of apoptosis, in sepsis *in vivo* and *in vitro*, suggesting that apoptosis in intestinal epithelial cells was induced by sepsis. NF-κB is thought to be a key regulator of inflammatory gene expression. NF-κB activation also plays an important role in the regulation of the expression of certain pro-inflammatory genes such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α. Importantly, we also noted that NF-κB activation was associated with the apoptosis of intestinal epithelial cells in sepsis. The relationship between NF-κB and apoptosis is

controversial. The direct effect of NF-κB activation has been reported to be inhibition of cell apoptosis, which is achieved through the expression of proteins in the inhibitors of apoptosis family. However, NF-κB can promote cell apoptosis by mediating the synthesis of inflammatory factors such as TNF-α and IL-6.[4] Therefore, the effect of NF-κB on apoptosis depends on the balance between the two processes. In our study, we found that inhibition of NF-κB partly rescued LPS-induced apoptosis in HT29 cells, indicating that NF-κB activation promotes cell apoptosis during sepsis. PLK1, as the most evolutionarily conserved member of the polo sub-family, is a cell cycle-related kinase required for proper M-phase progression and plays critical roles in diverse biochemical and cellular processes such as apoptosis and proliferation in humans.[5] Although PLK1 and NF-κB are both involved in apoptosis, the regulatory relationship between them is unclear. Our results illustrated that PLK1 activates NF-κB during sepsis, suggesting that PLK1 may be an upstream regulator of NF-κB.

In summary, sepsis induces intestinal epithelial cell apoptosis, and activation of NF-κB plays an important role in this process. Inhibiting NF-κB partially rescues the apoptosis induced by sepsis *in vitro*, and NF-κB activation is regulated by PLK1. Thus, the PLK1-NF-κB pathway might be critical in sepsis-induced intestinal barrier dysfunction.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Funding

This work was supported by grants from the Anhui Province Natural Science Foundation for Youth (No. 1908085QH360), and the "Peak" Training Program for Scientific Research of Yijishan Hospital, Wannan Medical College (Nos. GF2019J03 and GF2019G08).

Conflicts of interest

None.

References

1. Assimakopoulos SF, Triantos C, Thomopoulos K, Fligou F, Maroulis I, Marangos M, *et al*. Gut-origin sepsis in the critically ill patient: pathophysiology and treatment. Infection 2018;46:751–760. doi: 10.1007/s15010-018-1178-5.
2. Wullaert A, Bonnet MC, Pasparakis M. NF-kappaB in the regulation of epithelial homeostasis and inflammation. Cell Res 2011;21:146–158. doi: 10.1038/cr.2010.175.
3. Cao Y, Chen Q, Wang Z, Yu T, Wu J, Jiang X, *et al*. PLK1 protects against sepsis-induced intestinal barrier dysfunction. Sci Rep 2018;8:1055. doi: 10.1038/s41598-018-19573-x.
4. Kondylis V, Kumari S, Vlantis K, Pasparakis M. The interplay of IKK, NF-kappaB and RIPK1 signaling in the regulation of cell death, tissue homeostasis and inflammation. Immunol Rev 2017;277:113–127. doi: 10.1111/imr.12550.
5. de Carcer G, Manning G, Malumbres M. From Plk1 to Plk5: functional evolution of polo-like kinases. Cell Cycle 2011;10:2255–2262. doi: 10.4161/cc.10.14.16494.

How to cite this article: Cao YY, Wang Z, Lu LM, Xu ZX, Li JJ, Jiang XG, Lu WH. Polo-like kinase 1 protects intestinal epithelial cells from apoptosis during sepsis via the nuclear factor-κB pathway. Chin Med J 2020;133:1886–1888. doi: 10.1097/CM9.0000000000000780