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

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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. 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. 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 (Abcam), anti-PLK1 (Abcam), anti-pro-caspase-3 (Abcam), and anti-pro-caspase-9 (Abcam) antibodies, and results were evaluated blindly by two observers. 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 IkB-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 IkB-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. 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 IkB-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
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. 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. 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.

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Conflicts of interest

None.

References


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