Lipopolysaccharide Induces Disruption of the Tight Junction via Toll-Like Receptor 4 and Tumor Necrosis Factor Alpha

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Abstract：**Background & Aims:** Inflammatory bowel disease has been suggested to be resulted from a dysregulation of the innate response system and disruption of the epithelial barrier in the gut. Our aim was to investigate the functional response of changes in the barrier function of the intestinal epithelium by LPS via TLR4.

Methods: T84 cells, a human intestinal epithelial cell line, were cultured on the Transwell filters. LDH concentration in the culture medium was measured to assess cell viability for LPS. The cells were transfected with siRNA for TLR4. LPS was added to the apical side of the cells. The barrier function of the TJ was evaluated by measuring transepithelial electrical resistance (TER). The expressions of the TLR4 in T84 cells were examined by Western blotting and Real-Time RT-PCR analysis. TNFα secretion into the medium induced by LPS was measured by ELISA.

Results: TLR4 was expressed in T84 cells, and was upregulated by LPS. TLR4 siRNA significantly suppressed the TLR4 mRNA by 50-70% ($P < 0.001$). At the LPS concentration of 10, 100, 300 μg/ml, LDH was not increased compared with control. After 24 hours, TER was decreased by 16% at LPS 10 μg/ml ($P < 0.001$), 23% at 100 μg/ml ($P < 0.001$) and 40% at 300 μg/ml ($P < 0.001$). TLR4 siRNA prevented a decrease in TER in a lower concentration of LPS (10 μg/ml) ($P < 0.01$), but not in a higher concentration. LPS induced an increase in the secretion of TNFα in a dose-dependent manner ($P < 0.01$). TLR4 siRNA could prevent the secretion of TNFα only in a lower concentration of LPS (10 μg/ml) ($P < 0.01$).

Conclusions: TLR4 siRNA attenuates LPS-induced disruption of TJ and secretion of TNFα in T84 cells. Knockdown of TLR4 may be effective to prevent an increase in permeability from LPS.

Key words：**Tight junction, Toll-like receptor4, short-interfering RNA, Lipopolysaccharide, tumor necrosis factor alpha, T84 cell line**