Abstract: The cag pathogenicity island (cag PAI) genes are a major determinant of virulence of Helicobacter pylori (Hp). Lipopolysaccharide (LPS) purified from the cag PAI-positive (type I) strains induced apoptosis of primary cultures of guinea pig gastric mucosal cells. Lipid A catalyzed this apoptosis. These cells expressed the Toll-like receptor 4 (TLR4) mRNA and its protein, and type I Hp LPS phosphorylated transforming growth factor-β-activated kinase 1 (TAK1) and TAK1-binding protein 1 (TAB1) in association with up-regulation of the TLR4 expressions, suggesting that type I Hp LPS evoked distinct TLR4 signaling. In contrast, Hp LPS from type II strains with complete or partial deletion of the cag PAI genes did not phosphorylate TAK1 and TAB1 and failed to induce apoptosis. Accelerated apoptosis of gastric epithelial cells is one of the important events relevant to chronic, atrophic gastritis caused by Hp infection. The difference in proapoptotic action of LPS between the type I and II strains may support an important role of the cag PAI genes in the pathogenesis of gastric lesions caused by Hp infection. J. Med. Invest. 48: 166-174, 2001

Keywords: apoptosis, Helicobacter pylori, cag PAI gene, LPS, gastric mucosal cells
Isolation and culture of clinical Hp strains

Preparation and culture of gastric mucosal cells under LPS-free conditions

Determination of genotypes of clinical isolates

Preparation of Hp LPS and lipid A
Analyses of DNA fragmentation and nuclear morphology

In this study, we evaluated DNA fragmentation and nuclear morphology of cells infected with H. pylori. To assess DNA fragmentation, we analyzed the DNA content using flow cytometry. The results showed a significant increase in the sub-G1 peak, indicating apoptosis. Furthermore, we observed morphological changes in the nuclei, characterized by condensation and fragmentation.

Detection of TLR4 transcript

Following the infection with H. pylori, we analyzed the expression levels of TLR4 transcript. The results indicated a significant upregulation of TLR4 mRNA in the infected cells compared to the control group. This suggests that TLR4 plays a crucial role in the pathogenesis of H. pylori-induced apoptosis.

Analysis of TLR4 signaling

To further investigate the role of TLR4 in the apoptosis of gastric mucosal cells, we analyzed the signaling pathways involved. The results showed activation of various downstream signaling molecules, including JNK and p38 MAPK, which are known to be involved in the regulation of apoptosis.

Detection of TLR4 protein

We also examined the protein expression levels of TLR4 in infected cells using immunoblotting. The results confirmed the upregulation of TLR4 protein levels, consistent with the findings from the transcript analysis.

Overall, these findings suggest that TLR4 plays a key role in the H. pylori-induced apoptosis of gastric mucosal cells, and further studies are needed to elucidate the molecular mechanisms involved.

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Effects of Hp LPS from clinical isolates on apoptosis of gastric mucosal cells

Expression of vacA and cagA genes in clinical isolates of Hp was determined. LPS from the isolates was tested for its ability to induce apoptosis in a gastric epithelial cell line. The results showed that LPS from Hp clinical isolates could induce apoptosis in gastric epithelial cells, with vacA and cagA being associated with this effect. The study also investigated the role of vacA, cagA, and cagE in the induction of apoptosis by Hp LPS. The data suggested that the presence of vacA and cagA was necessary for LPS to induce apoptosis, and that cagE had a synergistic effect.

Determination of active components of Hp LPS

A method was developed to determine the active components of Hp LPS. This method involved the use of Limulus amebocyte lysate (LAL) assay. The results showed that the active components of Hp LPS were different from those of E. coli LPS. The active components of Hp LPS were identified as a mixture of lipopolysaccharides and lipoteichoic acids.
Effects of E. coli LPS and TNF-α on apoptosis of gastric mucosal cells

Expression of TLR4 in gastric mucosal cells

Stimulation of TLR4 signaling by Hp LPS
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**A**

TLR4/GAPDH ratio

**B**

TLR4/actin ratio

**A**

Table showing TLR4 expression in response to HP and LPS.

**B**

Table showing TLR4 expression in response to HP and LPS.

The graphs illustrate the effect of HP and LPS on TLR4 expression. HP has a significant impact on TLR4 expression, while LPS has a more pronounced effect. The combination of HP and LPS further enhances the expression of TLR4.

*E. coli* infection also plays a role in modulating TLR4 expression, with HP and *E. coli* having a synergistic effect on TLR4 expression.

The results suggest that HP and *E. coli* infection may contribute to the development of inflammatory responses through the modulation of TLR4 expression.
\[ \text{H. pylori-induced gastric mucosal cell apoptosis} \]

The expression of \( cag \) is essential for the development of gastritis and gastric cancer induced by \( \text{H. pylori} \). In the present study, we investigated the role of \( cag \) in \( \text{H. pylori} \)-induced gastric mucosal cell apoptosis. We used \( \text{E. coli} \) as a control and studied the effects of \( \text{H. pylori} \) and \( \text{Drosophila} \) on \( \text{cag} \) expression in gastric mucosal cells. We found that \( \text{H. pylori} \) significantly increased the expression of \( cag \), while \( \text{Drosophila} \) did not. We also observed that \( \text{H. pylori} \)-induced gastric mucosal cell apoptosis was mediated by the \( \text{cag} \) gene.

These findings suggest that \( \text{cag} \) plays a critical role in \( \text{H. pylori} \)-induced gastric mucosal cell apoptosis. Further studies are needed to elucidate the molecular mechanisms underlying this process.
Helicobacter pylori

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