Helicobacter pylori lipopolysaccharide induces apoptosis of cultured guinea pig gastric mucosal cells

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Helicobacter pylori lipopolysaccharide induces apoptosis of cultured guinea pig gastric mucosal cells. Am J Physiol Gastrointest Liver Physiol 281: G726–G734, 2001.—Helicobacter pylori lipopolysaccharide (LPS) is generally accepted as a low-toxicity virulence factor. Primary cultures of guinea pig gastric mucosal cells expressed the Toll-like receptor 4 and were sensitive to H. pylori LPS as well as Escherichia coli LPS. H. pylori LPS stimulated phosphorylation of transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1), and c-Jun NH2-terminal kinase (JNK). 2. H. pylori LPS at >2.1 endotoxin unit/ml (>1 ng/ml) activated caspase-8, stimulated cytochrome c release from mitochondria, and subsequently activated caspases-9 and -3, leading to apoptosis. Epidermal growth factor blocked all of these apoptotic processes and inhibited apoptosis, whereas it did not modify the phosphorylation of TAK1, TAB1, and JNK2. A comparatively specific inhibitor of caspase-8 or -9 blocked apoptosis, whereas cytochrome c release was prevented only with a caspase-8-like inhibitor. Our results suggest that caspase-8 and mitochondria may play crucial roles in H. pylori LPS-induced apoptosis and that this accelerated apoptosis may be involved in abnormal cell turnover of H. pylori-infected gastric mucosa.

Toll-like receptor 4; caspase-8; mitochondria; epidermal growth factor; gastric pit cells

APOPTOSIS IS DEFINED as programmed cell death characterized by distinct morphological and molecular features (12). It is essential not only for development and homeostasis of multicellular organisms (24, 39) but also for a defense mechanism against bacterial and viral infection (28). In chronic gastritis caused by Helicobacter pylori infection, accelerated apoptosis of gastric epithelial cells has been suggested to be associated with atrophy of gastric mucosa (33, 40). Activation of the Fas receptor (28), overproduction of tumor necrosis factor (TNF)-α (30), and increased expression of inducible nitric oxide synthase (21) or class II major histocompatibility complex (8) have been suggested to participate in the accelerated apoptosis observed in H. pylori infection. On the other hand, it is still unclear whether distinct bacterial factors are involved in this apoptosis. It was reported that soluble extracts of H. pylori stimulated apoptotic pathways (28, 40). Urease was then identified as one of the putative inducers (9).

Lipopolysaccharide (LPS) is a major membrane component of gram-negative bacteria and is composed of lipid A, inner core oligosaccharide, and oligosaccharide chains termed O antigen chains. H. pylori LPS has lower immunologic activities compared with those of Salmonella enterica or Escherichia coli, because 500–10,000 times higher concentrations of H. pylori LPS are required to induce 50% lethality of mice or to prime phagocytes (23, 27). On the other hand, H. pylori LPS was shown to stimulate histamine release and DNA synthesis in rat enterochromaffin-like cells more effectively than E. coli LPS (15). We also reported (35, 36) that H. pylori LPS could enhance production of superoxide anion from primary cultures of guinea pig gastric mucosal cells. These findings strongly suggest that H. pylori LPS has the ability to trigger gastric mucosal cell responses. However, the biological activities of H. pylori LPS and its intracellular signals are not fully understood.

In this study, we found that H. pylori LPS was a potent inducer for apoptosis of cultured guinea pig gastric mucosal cells. This apoptosis did not occur when epidermal growth factor (EGF) was present. On the basis of these findings, we examined H. pylori LPS-dependent apoptotic pathways and antiapoptotic actions of EGF on gastric mucosal cells.

MATERIALS AND METHODS

Preparation and culture of gastric mucosal cells under LPS-free conditions. Male guinea pigs weighing ∼250 g were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Gastric mucosal cells were isolated aseptically from guinea pig fundic glands as previously described (35). In the present experiments, all reagents used for culture were free from detectable amounts of LPS by Limulus amebocyte lysate assay (Endospect; Seikagaku Kogyo Tokyo, Japan). The isolated cells were cultured for 2 days in RPMI-1640 (GIBCO, Grand Island, NY) containing 50 μg/ml gentamicin, 100 U/ml penicillin G, and 10% (vol/vol) fetal bovine serum (FBS; ICN Biomedicals, Aurora, OH). This complete medium contained <0.01 endotoxin unit (EU)/ml of LPS. Cell populations were determined by histochemical and immuno-
hitochemical analyses as described previously (35). After culturing for 48 h, growing cells consisted of pit cells (~90%), pre-pit cells (~5%), parietal cells (4–5%), mucous neck cells (<1%), and fibroblasts (<1%).

Preparation and treatment of H. pylori LPS and lipid A. LPS was prepared from strain NCTC 11637 by the hot phenol-water method (35) and subsequently treated with DNase 1, RNase A, and proteinase K as described by Moran et al. (22). The treated LPS was boiled for 1 h to inactivate the enzymes. The LPS was dialyzed against LPS-free water (Otsuka Pharmaceutical, Tokushima, Japan) and ultracentrifuged at 100,000 g for 18 h. Precipitated LPS was dissolved in LPS-free saline (Otsuka Pharmaceutical). Lipid A was isolated by boiling the LPS in 0.1 M acetate buffer (pH 6.5) for 1 h (3) and then pelleted by centrifugation at 3,000 g for 30 min. Precipitated lipid A was dissolved in LPS-free saline.

Analyses of DNA fragmentation and nuclear morphology. Both floating and attached cells were lysed for 30 min at 4°C in 200 ml of 10 mM Tris-HCl buffer (pH 8.0) containing 10 mM EDTA and 0.5% (vol/vol) Triton X-100. Fragmented DNA of these samples was isolated and analyzed as described previously (37). In separate experiments, cells were fixed with 2% paraformaldehyde in PBS for 30 min at room temperature and were stained with the fluorescent dye Hoechst 33342 (Sigma Chemical, St. Louis, MO). Chromosomal condensation and fragmentation were examined with fluorescence microscopy as described previously (37).

Measurement of caspase activities. Cytosolic extracts were prepared, and caspase-3-like activity was measured as previously described (37). For measurement of caspase-8-like activity, the cytosolic extracts were mixed and incubated for 1 h at 37°C in 100 mM HEPES buffer, pH 7.5, containing 10% sucrose, 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml pepstatin, and the synthetic substrate N-Acetyl-Ile-Glu-Thr-Asp-p-nitroanilide (Ac-IETD-p-nitroanilide, 800 μM; Biomol Research Labs, Plymouth, PA). Caspase-8-like activity was assayed by measuring the increase in absorbance at 405 nm and was expressed as increase in absorbance per milligram of protein per hour. The reaction mixture without the synthetic substrate or cellular proteins was used as a negative control.

Preparation of mitochondria and cytosolic extracts. Cells were washed twice with ice-cold PBS, harvested with a rubber policeman, and collected in microcentrifuge tubes. The sample was separated into a mitochondria-free cytosolic extract and a mitochondrial pellet by the method of Bossy-Wetzel and Green (5). The levels of cytochrome c in both fractions were measured by immunoblot analysis.

Immunoblot analysis. After exposure to H. pylori LPS and/or EGF for the indicated times, cells were washed three times with saline, then treated with 21 endotoxin units (EU)/ml of H. pylori LPS or with saline (vehicle) in RPMI-1640 containing 0.1% fetal bovine (FBS) for the indicated times. C: H. pylori LPS (2.1 × 103 EU/ml) extracted by the hot phenol-water method was treated with 0.1 mg/ml DNase 1, 0.1 mg/ml RNase A, or 0.1 mg/ml proteinase K for 12, 4, or 4 h, respectively. These LPSs were boiled for 1 h to inactivate the enzymes. Cells were incubated with saline (lane 1) or one of these LPSs (21 EU/ml; lanes 2–4) for 8 h at 37°C. After H. pylori LPS (21 EU/ml) was incubated with different concentrations of polymyxin B for 1 h at 37°C, cells were treated with 21 EU/ml of H. pylori LPS for 8 h (lanes 5–8). D: lipid A was isolated as described in MATERIALS AND METHODS. Cells were incubated with different concentrations of lipid A for 8 h. These cells were harvested and analyzed for DNA ladder formation. Isolated DNA was subjected to 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Results were similar in 3 separate experiments.

Fig. 1. DNA ladder formation in gastric mucosal cells after treatment with Helicobacter pylori (Hp) lipopolysaccharide (LPS). A: after cultured gastric mucosal cells were washed with saline, they were treated with 21 endotoxin units (EU)/ml of Hp LPS or with saline (vehicle) in RPMI-1640 containing 0.1% fetal bovine (FBS) for the indicated times. B: cultured cells were treated with different concentrations of Hp LPS for 8 h. C: Hp LPS (2.1 × 103 EU/ml) extracted by the hot phenol-water method was treated with 0.1 mg/ml DNase 1, 0.1 mg/ml RNase A, or 0.1 mg/ml proteinase K for 12, 4, or 4 h, respectively. These LPSs were boiled for 1 h to inactivate the enzymes. Cells were incubated with saline (lane 1) or one of these LPSs (21 EU/ml; lanes 2–4) for 8 h at 37°C. After Hp LPS (21 EU/ml) was incubated with different concentrations of polymyxin B for 1 h at 37°C, cells were treated with 21 EU/ml of Hp LPS for 8 h (lanes 5–8). D: lipid A was isolated as described in MATERIALS AND METHODS. Cells were incubated with different concentrations of lipid A for 8 h. These cells were harvested and analyzed for DNA ladder formation. Isolated DNA was subjected to 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Results were similar in 3 separate experiments.
times with ice-cold PBS and lysed with 50 μl of 10 mM sodium phosphate buffer (pH 6.8) containing 2% SDS, 10% (vol/vol) glycerol, 2% (vol/vol) 2-mercaptoethanol, 25 mM dithiothreitol (DTT), 50 mM sodium fluoride, 10 μM trans-epoxysuccinyl-l-leucylamido-(4-guanidino)butane. Lysates were centrifuged at 15,000 g for 15 min at 4°C, and the resulting supernatants were collected. In separate experiments, cell proteins were extracted with 50 μl of 0.1 M Tris-Cl buffer (pH 8.0) containing 1 mM MgSO4 and the protease inhibitors and then treated with bacterial alkaline phosphatase (5 U/sample of a dish) for 60 min at 4°C. Each sample of 20 μg protein/lane was separated by SDS-PAGE and transferred to a polyvinylidene difluoride filter. After nonspecific binding sites were blocked with 4% purified milk casein, the filter was incubated for 1 h at room temperature with one of the following antibodies: antibodies against extracellular signaling-regulated kinase (Erk; Upstate Biotech., Lake Placid, NY), cytochrome c (PharMingen, San Diego, CA), human c-Jun NH2-terminal kinase (JNK) 1 (Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated JNK (Promega, Madison, WI), p90 ribosomal S6 kinase (Rsk) 2 and Bad (Transduction Labs, Lexington, KY), phosphorylated Rsk (Ser381) (New England Biolabs, Beverly, MA), phosphorylated Bad (New England Biolabs), and phosphorylated Erk (New England Biolabs), amino acid residues 554–579 of mouse transforming growth factor-β-activated kinase 1 (TAK1), amino acid residues 480–500 of human TAK1 binding protein 1 (TAB1), and Toll-like receptor 4 (TLR4). The polyclonal antibodies against TAK1 and TAB1 were gifts from Dr. K. Matsumoto (Nagoya University, Nagoya, Japan). The anti-TLR4 antibody was generated by immunization of a rabbit with synthetic peptide of amino acid residues 183–199 of human TLR4 (42). After being washed with PBS containing 0.05% (vol/vol) Tween 20, bound antibodies were detected by an enhanced chemiluminescence Western blotting detection system (Amersham Pharmacia, Piscataway, NJ). For immunoblot analysis with antibodies to caspase-8 (PharMingen) and -9 (StressGen Biotech., Victoria, BC, Canada), to avoid processing during preparation, whole cell proteins were extracted with an acid guanidinium thiocyanate-phenol-chloroform mixture (Isogen; Nippon Gene, Toyama, Japan) according to the manufacturer’s protocol. Each sample of 20 μg protein/lane was separated by SDS-PAGE in a 12% polyacrylamide gel, and immunoblot analysis was performed as described above. The bound antibodies were then removed by rinsing the membranes for 15 min at 50°C in 60 mM Tris-HCl buffer containing 0.1 mM 2-mercaptoethanol and 2% SDS. After being washed with PBS, the membrane was again subjected to immunoblotting with an antibody against actin (Oncogene Research, Cambridge, MA).
**Immunofluorescence histochemistry.** Cells growing on a LPS-free glass coverslip were washed with PBS and fixed with 4% paraformaldehyde in PBS for 20 min. After being washed with PBS, the cells were blocked with 4% purified milk casein. They were incubated with a 1:100 dilution of rabbit anti-TLR4 antibody for 1 h at room temperature and then treated with a 1:500 dilution of rhodamine-linked goat antibody against rabbit IgG (Amersham Pharmacia) for 1 h at room temperature. The samples were mounted with Vectashield mounting medium (Vector, Burlingame, CA). Subcellular localization of TLR4 was viewed using laser scanning confocal microscopy (model TCSNT; Leica, Heidelberg, Germany).

**Statistical analysis.** ANOVA and Scheffé’s test were used to determine statistically significant differences. Differences were considered significant if \( P < 0.05 \).

**RESULTS**

**Apoptosis of gastric mucosal cells after treatment with H. pylori LPS.** As shown in Fig. 1A, treatment of gastric mucosal cells with 21 EU/ml of H. pylori LPS caused DNA ladder formation within 5 h. When cells were incubated with different concentrations of H. pylori LPS for 8 h, H. pylori LPS at \( \geq 2.1 \) EU/ml (1 ng/ml) induced DNA fragmentation (Fig. 1B). H. pylori LPS extracted by the hot phenol-water method was treated with DNase 1, RNase A, or proteinase K, but none of these enzymes removed the activity of H. pylori LPS (Fig. 1C). Polymyxin B inhibited H. pylori LPS-induced ladder formation (Fig. 1C), and lipid A was confirmed to be a bioactive component of H. pylori LPS (Fig. 1D). We also confirmed that E. coli LPS (K235 strain; Sigma Chemical) at >0.3 EU/ml (>1 ng/ml) similarly induced apoptotic DNA fragmentation (data not shown).

Treatment with 21 EU/ml of H. pylori LPS for 8 h significantly increased the number of apoptotic cells (Fig. 2B). When EGF was included, EGF at \( \geq 5 \) nM suppressed LPS-induced apoptosis in a dose-dependent manner and 20 nM EGF completely inhibited DNA ladder formation (Fig. 2A) and apoptosis (Fig. 2B).

**Expression of TLR4 in gastric mucosal cells.** Among the TLR family members, TLR4 is now identified as the specific receptor for LPS in vivo and in vitro (34).
Human and guinea pig neutrophils (PMN) were isolated as previously described (16, 35). Immunoblot analysis with an anti-TLR4 antibody showed that guinea pig gastric mucosal cells expressed TLR4 protein as was detected in human and guinea pig PMN (Fig. 3A). Immunocytochemical analysis showed that TLR4 was preferentially distributed on plasma membrane (Fig. 3B).

Activation of TLR4-signaling pathway. TAK1 is a member of the mitogen-activated protein (MAP) kinase kinase kinases that activate MAP kinase cascades, including JNKs (32). TAK1 is now recognized as one of the common signal transduction molecules for TLR4- and interleukin 1 receptor (IL-1R)-signaling pathways (14, 26). TAB1 functions as an activator for the TAK1 (31). We tested whether \textit{H. pylori} LPS activates these signal-transducing molecules. Treatment with \textit{H. pylori} LPS rapidly phosphorylated TAK1, TAB1, and JNK2 (Fig. 4, left). In contrast, EGF phosphorylated only JNK1 (Fig. 4, right). The presence of EGF did not modify the \textit{H. pylori} LPS-induced phosphorylation of TAK1, TAB1, and JNK2 (Fig. 4, middle).

EGF-induced survival signals. Although EGF did not block the LPS-induced activation of TAK1, TAB1, and JNK2, EGF activates multiple cascades. In particular, EGF-induced activations of phosphatidylinositol 3-kinase (10, 17), protein kinase C (17), protein kinase B (10), and Erk (4) have been considered to be important survival signals. We observed that PD-98059, which inhibits Erk1 and -2, triggered apoptosis of gastric mucosal cells (data not shown), suggesting that EGF might block the \textit{H. pylori} LPS-induced apoptosis by activating an Erk-dependent pathway. In fact, EGF phosphorylated Erk2 within 10 min (Fig. 5A, right).

Fig. 5. Analysis of EGF signals. Cultured cells were treated with 21 EU/ml \textit{Hp} LPS (lanes 1–6 in A and B and lanes 1–8 in C), 21 EU/ml \textit{H. pylori} LPS + 20 nM EGF (lanes 7–12 in A and B and lanes 9–16 in C), or 20 nM EGF (lanes 13–18 in A and B and lanes 17–24 in C) in RPMI-1640 containing 0.1% FBS for the indicated times. Extracted proteins were subjected to SDS-PAGE using 10% (for Erk), 6% (for Rsk) and 12% (for Bad) polyacrylamide gels, and immunoblot analysis with an antibody against phosphorylated Erk (Thr202/Tyr204), Erk, phosphorylated Rsk (Ser381), Rsk2, phosphorylated Bad (Ser112), or Bad was performed as described in Fig 4. p-Erk1 or 2, phosphorylated Erk1 or -2; p-Rsk, phosphorylated Rsk; p-Bad, phosphorylated Bad. Similar results were obtained in 3 separate experiments.

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This phosphorylation did not occur in the presence of \textit{H. pylori} LPS (Fig. 5A, middle). Erk1 or -2 activates several antiapoptotic proteins, such as MAP kinase-activated protein kinase-1 (also known as Rsk), Bad, and cAMP response element-binding protein (CREB) (4). In gastric mucosal cells, EGF phosphorylated Rsk and Bad (Fig. 5, B and C, right). These sequential phosphorylations were also documented in cells simultaneously treated with \textit{H. pylori} LPS and EGF (Fig. 5, B and C, middle) but not in cells treated with \textit{H. pylori} LPS alone (Fig. 5, B and C, left). We also confirmed that EGF and \textit{H. pylori} LPS did not significantly change the level of Bcl-2 (data not shown).

Effects of \textit{H. pylori} LPS on cytochrome c release. As described above, \textit{H. pylori} LPS evoked TLR4-mediated signals and EGF triggered distinct survival signals. To reveal how \textit{H. pylori} LPS induced apoptosis and how EGF prevented this apoptosis, we focused on mitochondria. As shown in Fig. 6A, \textit{H. pylori} LPS triggered cytochrome c release from mitochondria within 4 h, and this release was completely blocked by EGF. Comparatively specific inhibitors of caspase-9 (acetyl-Leu-Glu-His-Asp-aldehyde; Ac-LEHD-CHO) or caspase-3 (acetyl-Asp-Met-Gln-Asp-aldehyde; Ac-DMQD-CHO) did not prevent the release induced by \textit{H. pylori} LPS, suggesting that the release of cytochrome c was not a secondary response induced by activated downstream caspases such as caspase-3 (Fig. 6B). It should be noted that a caspase-8-like inhibitor [acetyl-Ile-Glu-Thr-Asp-aldehyde (Ac-IETD-CHO)] prevented cytochrome c release (Fig. 6B).

Effects of \textit{H. pylori} LPS on caspases. A broad-spectrum caspase inhibitor (z-Val-Ala-Asp-CH$_2$F; z-VAD-fmk) (Fig. 7A) and a caspase-9-like inhibitor (Ac-LEHD-CHO) (Fig. 7B) inhibited apoptotic DNA fragmentation induced by \textit{H. pylori} LPS in a dose-dependent manner. As shown in Fig. 7C, \textit{H. pylori} LPS-induced DNA fragmentation was more sensitive to inhibition by Ac-IETD-CHO than by Ac-LEHD-CHO and z-VAD-fmk; the fragmentation was suppressed with Ac-IETD-CHO at lower concentrations than those of the other two inhibitors.
After treatment with *H. pylori* LPS, the cleavage of pro-caspase-9 (Fig. 8C, left) and elevation of caspase-3-like activity (Fig. 8A) were observed at 8 h. On the other hand, pro-caspase-8 was cleaved within 4 h (Fig. 8D, left) and caspase-8-like activity increased 2–4 h after the treatment (Fig. 8B). We also confirmed that EGF completely prevented the processing of pro-caspases-8 and -9 and significantly suppressed the elevations of caspase-8- and 3-like activities (Fig. 8, A and B).

**DISCUSSION**

Using LPS-free cultures of guinea pig gastric mucosal cells, we demonstrated that gastric mucosal cells were extremely sensitive to *H. pylori* LPS as well as *E. coli* LPS; both *H. pylori* LPS (>1 ng/ml) and *E. coli* LPS (>1 ng/ml) induced apoptosis of the cells. Recently, Cario et al. (7) showed that intestinal epithelial cells are relatively insensitive to *E. coli* LPS; ≥5 μg/ml concentrations of *E. coli* LPS were required to stimulate distinct signals of TLR4 expressed on intestinal epithelial cells. Primary cultures of gastric mucosal cells expressed the TLR4 mRNA and protein, and significant amounts of the receptor protein were distributed on plasma membrane. In phagocytes, LPS stimulates TLR4 signal transduction molecules such as myeloid differentiation factor 88 (MyD88), IL-1R-associated kinase, TNF receptor-associated factor 6, and TAK1 (14). Low concentrations of *H. pylori* LPS actually phosphorylated TAK1 and TAB1, showing that *H. pylori* LPS was able to stimulate TLR4-signaling cascades in gastric mucosal cells.

In association with the activation of TAK1 and TAB1, *H. pylori* LPS phosphorylated JNK2. At first, we thought that JNK2 was a possible protein to mediate apoptosis because phosphorylation of JNK1 or -2 is one of the essential events triggering the release of death factors from mitochondria in neurons (38). However, EGF inhibited cytochrome *c* release without affecting phosphorylation of TAK1 and JNK2 induced by *H. pylori* LPS. EGF phosphorylated JNK1. JNKs have dual effects that are apparently contradictory (19): JNK-catalyzed phosphorylation of c-Jun is one of the important signals for proliferation and transformation of cells (29), whereas overexpression of c-Jun results in apoptosis in fibroblasts or neuronal cells (6, 18). It is possible to speculate that JNK1 may exhibit an anti-apoptotic action, but there is no evidence to support this hypothesis.

EGF promotes cell survival through an Erk-Rsk signaling pathway, leading to phosphorylation of Bad and CREB (4). Phosphorylated CREB functions as a transcription factor that regulates expression of the anti-apoptotic gene *bcl-2* (41). However, we found that EGF did not change the level of Bcl-2 protein in gastric mucosal cells (data not shown). EGF phosphorylated Erk2, Rsk, and Bad in these cells. The different signals converge in mitochondria to trigger or inhibit cytochrome *c* release. Once cytochrome *c* is released, it forms an essential part of the apoptosome, which in turn activates pro-caspase-9 in collaboration with apoptotic protease-activating factor-1, resulting in the activation of caspase-3 (11). Comparatively specific inhibitors of caspases-9 and -3 did not prevent the *H. pylori* LPS and gastric mucosal cell apoptosis.
LPS-induced cytochrome c release, suggesting that these downstream caspases were not involved in the mitochondrial dysfunction. Finally, we found that a caspase-8-like inhibitor not only prevented cytochrome c release but also blocked apoptosis at the lowest concentration among the caspase inhibitors tested. After treatment with *H. pylori* LPS, the cleavage of procaspase-8 and elevation of caspase-8-like activity occurred before processing of pro-caspase-9 and activation of caspase-3-like proteases. These results suggest that caspase-8 may be a crucial molecule to induce cytochrome c release and to activate downstream caspases.

In addition to caspase-3-activating capability, caspase-8 has been shown to stimulate cytochrome c release directly by cleavage of Bid, a Bcl-2-related protein (20). Considering that a comparatively specific inhibitor of caspase-9 alone also prevented apoptotic DNA fragmentation, caspase-8-mediated mitochondrial dysfunction may be one of the major routes to apoptosis induced by *H. pylori* LPS.

Caspase-8 is a crucial mediator of apoptosis induced by bacterial lipoproteins in the TLR2-dependent apoptotic pathway as well as Fas ligand or TNF-α (1, 2, 24). Primary cultures of guinea pig mucosal cells did not contain measurable numbers of immune cells (35), and recombinant TNF-α did not induce apoptosis of these cells (data not shown). We also confirmed that the cultured cells did not express the TLR2 transcript or recombinant LPS in this study. MyD88 is a crucial adaptor protein of TLRs, and it contains a homologous sequence termed the death domain. On recruitment of Fas-associated death domain to MyD88, caspase-8 oligomerization drives its activation through self-cleavage (2). The importance of the caspase-8-mediated pathway was indirectly supported by the fact that EGF also blocked this pathway.

Recently, it was shown that MAPK/Erk signaling inhibits the Fas-mediated activation of caspase-8 and Bid in activated T cells (13). EGF might interfere with *H. pylori* LPS-induced apoptosis through Erk signaling. However, EGF activates a number of proteins that exert antia apoptotic properties, and these antia apoptotic proteins triggered by EGF have not been shown directly to prevent apoptosis by *H. pylori* LPS in this system. Thus further experiments are necessary to elucidate the molecular basis of our findings; however, our results suggest that *H. pylori* LPS-induced apoptosis may participate in disintegrated cell turnover observed in gastric mucosa of subjects with *H. pylori* infection.

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