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Apoptotic Signaling Pathway Activated by *Helicobacter pylori* Infection and Increase of Apoptosis-Inducing Activity under Serum-Starved Conditions

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The enhanced gastric epithelial cell apoptosis observed during infection with *Helicobacter pylori* has been suggested to be of significance in the etiology of gastritis, peptic ulcers, and neoplasia. To investigate the cell death signaling induced by *H. pylori* infection, human gastric epithelial cells were incubated with *H. pylori* for up to 72 h. *H. pylori* infection induced the activation of caspase -8, -9, and -3 and the expression of the proapoptotic Bcl-2 family proteins Bad and Bid. The peak of the activity of the caspases occurred at 24 h. At this time, the inhibition of caspase-8 or -9 almost completely suppressed *H. pylori*-induced apoptosis. Inhibition of caspase-8 suppressed the expression of Bad and Bid and the subsequent activation of caspase-9 and -3. These observations indicate that *H. pylori* induces apoptosis through a pathway involving the sequential induction of apical caspase-8 activity, the proapoptotic proteins Bad and Bid, caspase-9 activity, and effector caspase-3 activity. Activation of the pathway was independent of CagA or vacuolating toxin. A membrane fraction of *H. pylori* was sufficient to activate this pathway, and treatment with proteinase K eliminated the activity. Apoptotic activity of the membrane fraction was significantly increased by incubating the bacteria under serum-starved conditions for 24 h. These observations suggest that environmental conditions in the human stomach could induce *H. pylori*-mediated pathogenesis, leading to a variety of clinical outcomes.

Helicobacter pylori is a human-specific gastric pathogen that colonizes the stomachs of at least half the world's population (7, 19, 21). Infection with *H. pylori* is strongly associated with gastric atrophy, peptic ulceration, and gastric cancer (8, 15, 18, 49, 59). *H. pylori* attaches to the gastric epithelium and exerts its pathogenic actions on the defense system responsible for the maintenance of mucosa homeostasis (37).

Apoptosis, programmed cell death, plays an important role in the regulation of epithelial cell numbers in the gastrointestinal tract (30). Deregulation of the apoptotic pathway is implicated in a number of disease processes in the gastrointestine (60). In *H. pylori*-induced chronic gastritis, cell loss by apoptosis is excessive compared with proliferation (54). In vivo studies demonstrate that infection with *H. pylori* triggers apoptosis of gastric epithelial cells (53). It is suggested that acceleration of apoptosis plays an important role in *H. pylori*-mediated pathogenesis (14, 28, 36, 42, 43).

To date, a number of studies have investigated the pathogenicity of *H. pylori* in relation to cytotoxic products, including urease, Cag, and vacuolating toxin (VacA). Recently potential apoptosis-inducing activity was reported in VacA (26) and urease (24). However, this viewpoint has often failed to elucidate the diverse pathomorphisms in *H. pylori* infections. Thus, it has been suggested that it is important to investigate the host factors which might affect cellular responses that would be involved in the development of gastric mucosal disorders. El-Omar et al. accordingly reported that interleukin 1 gene cluster polymorphisms suspected of enhancing the production of interleukin 1β are associated with an increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer (22). Apoptosis in *H. pylori*-associated gastritis accompanies the activation of Fas and the Fas ligand system (34, 61, 73) in epithelial cells. Fas is a member of the tumor necrosis factor receptor family, which, when bound by its ligand, activates caspase-8, an initiator of the downstream apoptotic process that includes the cleavage of other death substrates, cellular and nuclear morphological changes and, ultimately, cell death (56, 62, 70). The inflammatory mediators gamma interferon (IFN-γ) and tumor necrosis factor alpha augment apoptosis induced by *H. pylori* (72). Variations in host responses including these inflammatory mediators might cause the *H. pylori*-mediated pathogenesis to result in a variety of clinical outcomes.

Recent studies indicate that many apoptotic responses are initiated by activation of the apical caspase-8 or caspase-9: the former by the tumor necrosis factor receptor family (11, 55) and the latter by the release of cytochrome *c* following mitochondrial damage (66, 78). Activation of either of these two initiator caspases can lead to activation of the effector caspase-3 (67, 70).

In this study we explored the involvement of these signal pathways in *H. pylori*-induced apoptosis and the influence of growth conditions on the apoptosis-inducing activity of *H. pylori*.

MATERIALS AND METHODS

Bacterial strains, cell lines, and media. *H. pylori* strains NGY 273, isolated from a 61-year-old female with atrophic gastritis, NGY 621, from a 64-year-old male with gastritis, NGY 1268, from a 56-year-old male with early gastric cancer (type IIc), and NGY 1281, from a 53-year-old male with a gastric ulcer, were

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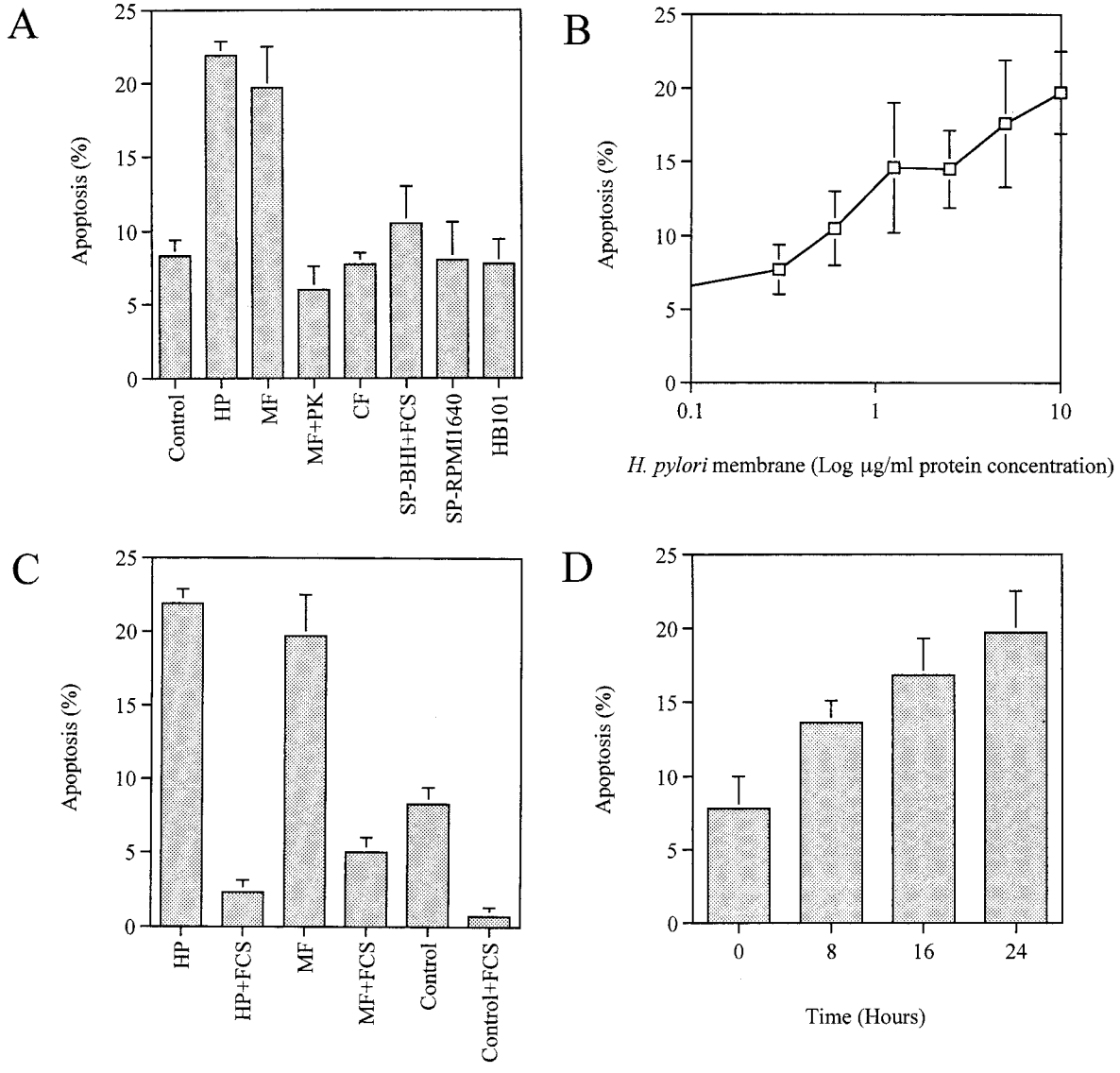

FIG. 1. Quantitation of *H. pylori*-induced apoptosis by fluorescence microscopy. Since there was no significant difference among the strains examined, data from NGY 1281 are shown as representative. Results are expressed as the mean percentages of apoptotic cells per 500 cells enumerated. Bars represent mean values ± SD from three experiments. (A) Induction of apoptosis by live *H. pylori* (HP), *H. pylori* membrane fraction (MF), membrane fraction pretreated with proteinase K (MF+PK), *H. pylori* cytosol (CF), culture supernatant from BHI broth with FCS (SP-BHI+FCS), culture supernatant from RPMI 1640 medium (SP-RPMI 1640), and *E. coli* HB101 (HB101). (B) Dose-dependent induction of apoptosis by the membrane fraction preparation. (C) Effect of serum on *H. pylori*-mediated apoptosis. (D) Time-dependent increase in apoptotic activity of the membrane fraction during incubation in serum-free RPMI 1640 medium. Membrane fraction preparations were added at a protein concentration of 10 μg/ml.

used. These strains were freshly isolated from biopsy specimens and stored at −80°C in brain-heart infusion (BHI) broth containing 10% fetal calf serum (FCS) and 15% glycerol. The strain NGY 273 was *cagA* negative and vacuolating toxin negative, while the other strains were *cagA* positive and vacuolating toxin positive. The presence of *cagA* was confirmed by PCR with the primer pair 5′-GGCAATGGTGGTCCTGGAGCTAGGC-3′ (nucleotides 1495 to 1519 in *cagA*) and 5′-GGAAATCTTTAATCTCAGTTCGG-3′ (nucleotides 1797 to 1819 in *cagA*). The presence of VacA was determined by the method of Cover et al. (16). Concentrated broth supernatants were incubated with HeLa cells (CRL-JCRB9004; Health Science Research Resource Bank, Osaka, Japan) for 24 h at 37°C, and vacuolation was assessed by bright-field microscopy.

H. pylori strains were grown on 7% horse blood agar plates at 37°C under microaerophilic conditions. Fresh plates were started from the glycerol stocks each week and passaged after 48 h. Liquid cultures of *H. pylori* were grown in BHI broth supplemented with 10% FCS under the same conditions for 24 h with agitation. The human gastric adenocarcinoma cell line AGS was obtained from American Type Culture Collections (CRL-1739) and maintained in Ham's F12 medium with 10% FCS. Cells were serum starved for 16 h and incubated with *H. pylori* at a bacterium/cell ratio of 100:1 for up to 72 h in the medium without serum.

Preparation of cellular membrane fraction from *H. pylori*. The bacteria grown in BHI broth with 10% FCS were harvested, washed with phosphate-buffered saline (PBS), and resuspended in serum-free RPMI 1640 medium. This bacterial suspension was incubated for 24 h unless otherwise stated. Then cells were harvested, washed, and resuspended in precooled PBS (4 ml per 100 ml of original culture). Cells were disrupted by one passage through a French pressure cell at 120 MPa. After low-speed centrifugation (5000 × *g*; 30 min) to remove cellular debris and unbroken cells, cellular membrane was sedimented by cen-

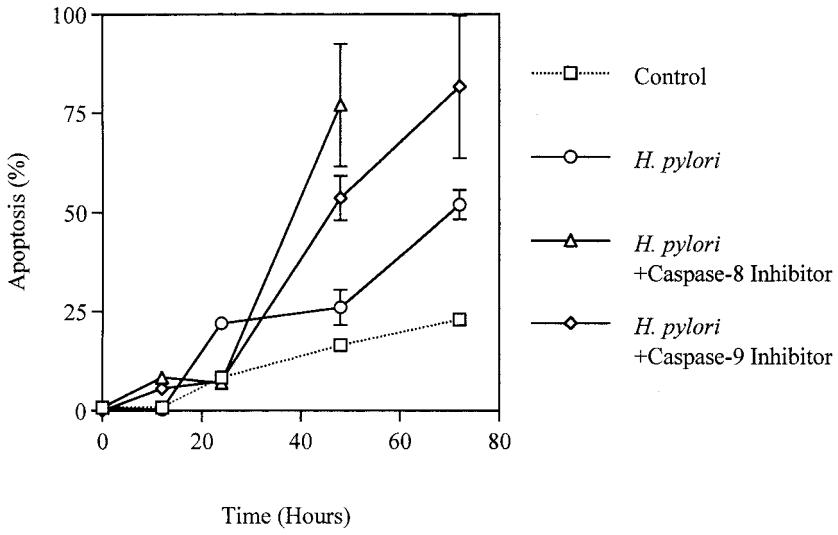

FIG. 2. Time course of *H. pylori* NGY 1281-induced apoptosis in AGS cells in the absence or presence of caspase inhibitors. Results are expressed as the mean percentages of apoptotic cells per 500 cells enumerated. Bars represent mean values ± SD from three experiments.

trifuging at 100,000 × *g* for 1 h at 4°C. The cellular membrane was resuspended in PBS to a protein concentration of 1 mg/ml. The supernatant was used as the cytosolic fraction. Where indicated, the membrane preparation was digested with proteinase K (Wako) at a concentration of 1 μg/ml at 50°C for 3 h. The membrane fraction was then sedimented by centrifuging at 100,000 × *g* for 1 h at 4°C to remove the proteinase K.

Reagents. Caspase-8 inhibitor, Z-IETD-FMK, and caspase-9 inhibitor, Z-LEHD-FMK, were purchased from Calbiochem (San Diego, Calif.). Antibodies to Bad and Bid were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.). Antibodies to phosphorylated Bad at serine-112 or -136 were from New England BioLabs, Inc. (Beverly, Mass.).

Assessment of apoptosis. Cells in suspension and trypsinized cells were pelleted and resuspended in PBS. Then cells were incubated with 100 nM Hoechst 33342 (Molecular Probes, Eugene, Oreg.) for 5 min at room temperature. A drop of the suspension was applied to a microscope slide, and apoptotic cells were assessed by fluorescence microscopy. Nuclei with highly condensed and fragmented chromatin were considered apoptotic. Apoptotic cells were enumerated by counting 500 cells in multiple randomly selected fields. The apoptotic index was expressed as the percentage of apoptotic cells per 500 cells enumerated.

Caspase activity assay. The activies of the apical caspase-8 and -9 and effector caspase-3 were determined using Caspase Colorimetric Protease Assay kits (Medical & Biological Laboratories, Nagoya, Japan) according to the manufacturer's instructions.

Western blotting. Cells were lysed in a buffer containing 1% Triton X-100, 10 mM Tris-HCl, pH 7.4, and protease inhibitors, and the resulting insoluble material was removed by centrifugation. For the analysis of cytosolic proteins, a digitonin permeabilization technique (33) was used to release cytosol from cells. Fifty-microgram protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, Calif.), and immunoblotted overnight with antibodies at a concentration of 1:1,000 (vol/vol). Immunocomplexes were visualized by enhanced chemiluminescence detection (ECL; Amersham Pharmacia Biotech, Uppsala, Sweden) using horseradish peroxidase-conjugated secondary antibodies following the protocol provided by the manufacturer.

Statistical analyses. Results are expressed as the means ± the standard deviations (SD). Induction of apoptosis and activities of caspases were compared using a two-tailed Student *t* test and considered significant if the *P* values were <0.01.

RESULTS

Evaluation of *H. pylori*-induced apoptosis. Quantitation of apoptotic AGS cells by fluorescence microscopy demonstrated that incubation with *H. pylori* induced a significant increase in apoptosis compared to the apoptosis of untreated cells at 24 h (21.9% ± 1.0% versus 8.3% ± 1.1%; $P < 0.01$). No significant difference in the level of apoptosis was observed among the strains examined, suggesting that apoptosis was independent of the secretory proteins CagA and VacA. A membrane fraction of *H. pylori* at a protein concentration of 10 μg/ml elicited a comparable response (Fig. 1A). Apoptosis was completely blocked by pretreating the membrane fraction preparation with proteinase K, indicating the involvement of an *H. pylori* membrane protein(s) in the initiation of apoptotic signaling. Induction of apoptosis by the membrane fraction preparation was dose dependent (Fig. 1B). Neither the cytosolic fraction nor the concentrated culture supernatant from BHI broth or RPMI 1640 medium induced apoptosis, nor did *Escherichia coli* HB101 induce apoptosis of AGS cells (Fig. 1A). The apoptotic effect of *H. pylori* was significantly suppressed by the addition of serum (21.9% ± 1.0% versus 2.3% ± 0.8% by live bacteria; 19.7% ± 2.8% versus 5.0% ± 1.0% by membrane fraction; $P < 0.01$) (Fig. 1C). Moreover, transfer of the bacteria grown in BHI broth with FCS to serum-free RPMI 1640 medium induced a time-dependent increase in the apoptotic activity of the membrane fraction (Fig. 1D). At 0 h, apoptosis-inducing activity was no more than the control level (7.8% ± 2.2% versus 8.3% ± 1.1%), while at 24 h it had increased to a level comparable to that elicited by the live bacteria under the serum-deprived conditions (19.7% ± 2.8% versus 21.9% ± 1.0%). These results indicate an antiapoptotic effect of serum on both AGS cells and the bacteria. Hence, the following analyses of *H. pylori*-induced apoptosis were performed using serum-deprived AGS cells as described in Materials and Methods unless otherwise stated.

Caspase activation and effect of caspase inhibitors on *H. pylori*-induced apoptosis. Coculture of AGS cells with *H. pylori* induced apoptosis in a time-dependent manner (Fig. 2). The activities of caspase-8, -9, and -3 were significantly elevated in *H. pylori*-infected AGS cells, with the peak occurring at 24 h, compared with control AGS cells (fold activation, for caspase-8, 7.2 ± 0.9 versus 4.1 ± 0.7; for caspase-9, 4.3 ± 0.1

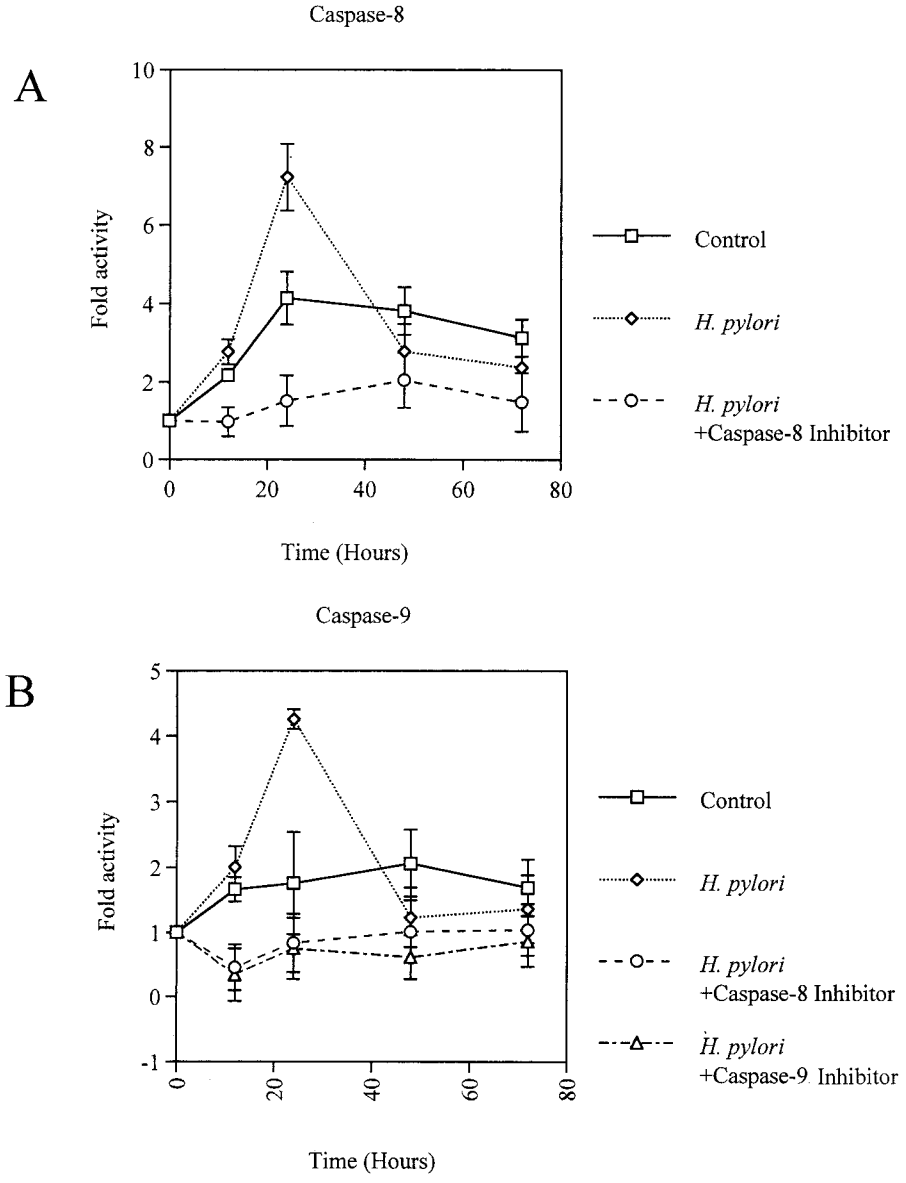

FIG. 3. (A to C) Activation of caspase-8 (A), -9 (B), and -3 (C) in *H. pylori* NGY1281-infected AGS cells. Effects of the inhibitors of caspase-8 and -9 were also examined. (D) Dose-dependent activation of caspase-8, -9, and -3 by *H. pylori* membrane fraction at 24 h. AGS cells were exposed to the *H. pylori* membrane preparation at the indicated protein concentration for 24 h. Bars represent mean values ± SD from three experiments.

versus 1.8 ± 0.8; for caspase-3, 4.8 ± 0.6 versus 3.6 ± 0.2; $P < 0.01$) (Fig. 3A, B, and C). These responses were also elicited by the membrane fraction preparations of *H. pylori*. Activation of the caspases by the membrane fractions was dose dependent (Fig. 3D). Treatment of the preparations with proteinase K eliminated the activity. To determine the contribution of caspase-8 and -9 to the activation of caspase-3, AGS cells were incubated with *H. pylori* in a medium containing 40 μM caspase-8 inhibitor Z-IETD-FMK or 20 μM caspase-9 inhibitor Z-LEHD-FMK. As Fig. 3C shows, caspase-3 activation was completely inhibited by both of these caspase inhibitors during the entire time course examined. In addition, caspase-8 inhibition also blocked the activation of caspase-9 (Fig. 3B). These results indicate a crucial role of caspase-8 and -9 in the activation of effector caspase-3 and a cascade process of their sequential activation in *H. pylori*-infected AGS cells. No significant difference was observed among the strains examined. At 24 h, the apoptosis was almost completely blocked by inhibition of either caspase-8 or -9 (21.9% ± 0.95% versus 6.9% ± 1.1% and 7.4% ± 1.3%, respectively; $P < 0.01$) (Fig. 2), indicating that these caspases play a critical role at this time point. However, these caspase inhibitors did not suppress the apoptosis beyond that time (Fig. 2), suggesting an involvement of caspase-independent pathways in the late phase, when a relatively high degree of apoptosis is induced. Similar phenomena were elicited by the membrane fraction preparations from *H. pylori* (data not shown). These observations indicate that caspase-8, -9, and -3 exert their apoptotic function at an early phase, when activities of these caspases attain relatively high levels.

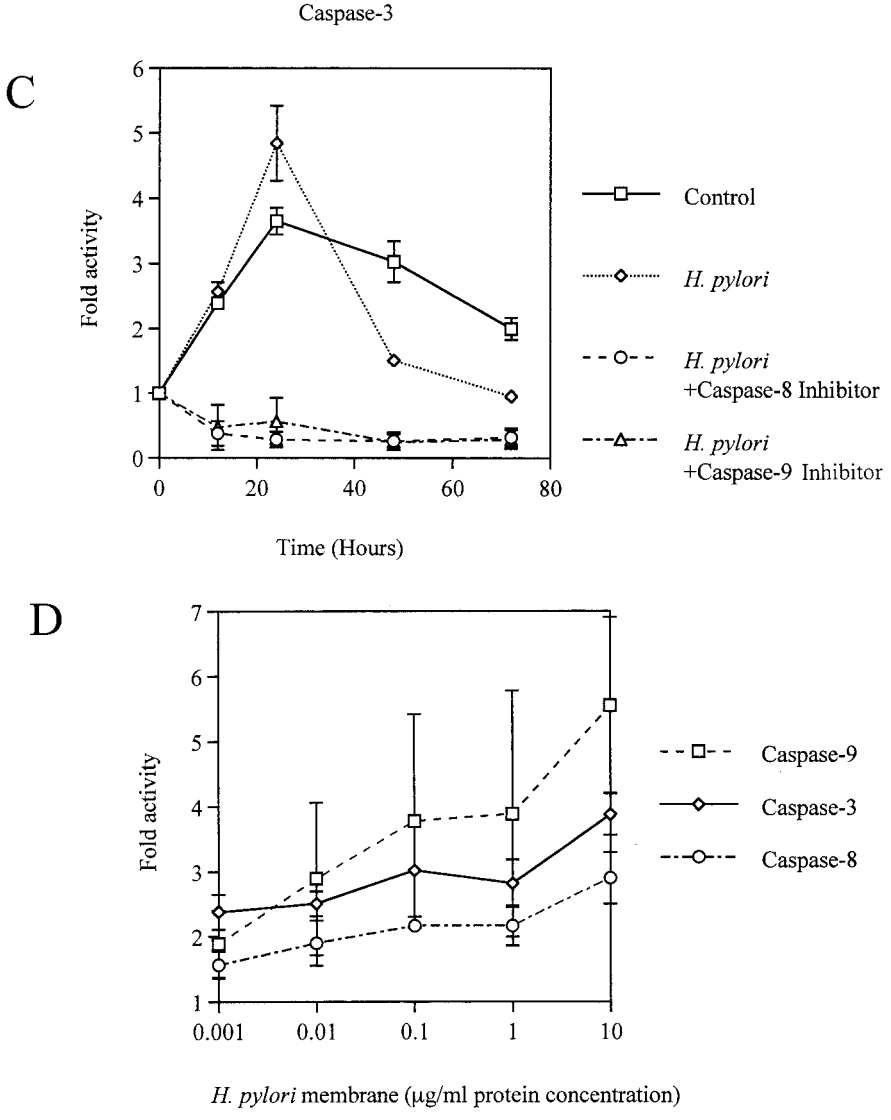

FIG. 3—*Continued.*

Expression of Bad and Bid during *H. pylori* infection. Since the activation of caspase-9 takes place by the release of cytochrome *c* following mitochondrial damage (66, 78), we investigated the expression of the proapoptotic Bcl-2 family member proteins Bad and Bid, which potentially exert their proapoptotic activity in mitochondria to cause a disruption of the mitochondrial membrane (74, 75). Bad and Bid were barely detectable in control AGS cells, but after the incubation with *H. pylori*, there was a significant increase in the expression of these proteins (Fig. 4A). No apparent difference was observed among the strains examined. As expected, the membrane preparations of *H. pylori* elicited the same response (Fig. 4B). The proteinase K-treated membrane preparations, cytosolic fraction, and concentrated supernatant showed no activity in inducing the expression of these proteins, nor did *E. coli* HB101 induce these proteins (Fig. 4B). Bad phosphorylated at either Ser-112 or Ser-136, whose proapoptotic activity was lost by an alteration in the subcellular location from mitochondria to cytosol (76), was not detected at any time between 12 and 72 h (not shown) during *H. pylori* infection. The Bid protein detected was a 13-kDa protein which was reported to possess mitochondrial damage-inducing activity (45). Moreover, Bad and Bid were not detected in the cytosolic extracts of AGS cells (data not shown). These observations suggest that Bad and Bid exert their death-promoting effects in the mitochondria of *H. pylori*-infected AGS cells. Inhibition of caspase-8 suppressed the expression of Bad and Bid between 12 and 24 h (Fig. 4C). This suggests that the induction of the proapoptotic proteins Bad and Bid at the early phase requires caspase-8 activity. In the late phase, alternative factors seem to induce the expression of Bad and Bid, which does not lead to the activation of caspase-9. These findings are consistent with the results obtained by a caspase activity assay showing the inhibitory effect of the caspase-8 inhibitor on the activation of caspase-9. In contrast, the inhibition of caspase-9 did not notably affect the expression of these proteins (Fig. 4D). This is in agreement

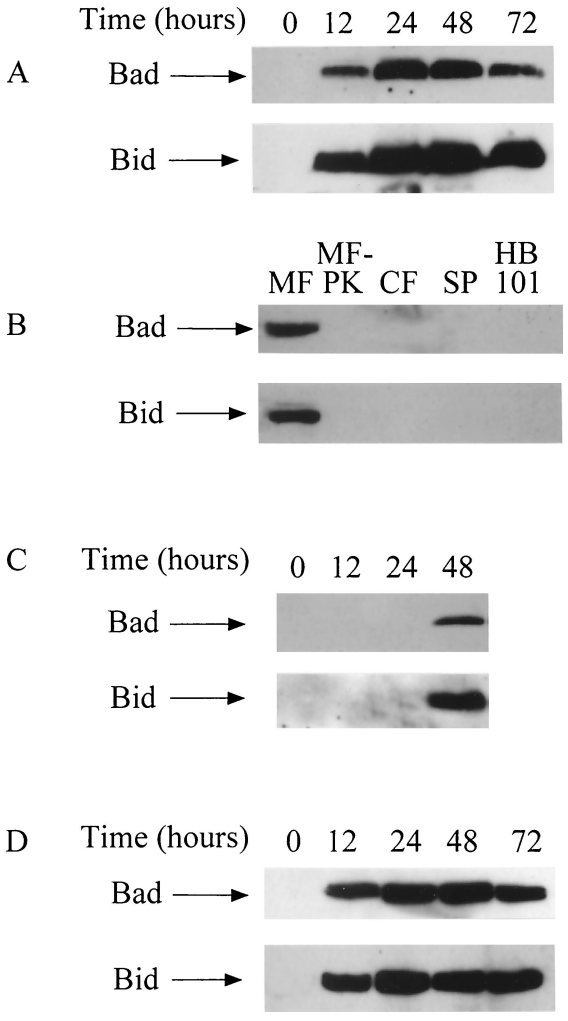

FIG. 4. Western blot analysis for Bad and Bid. Data from NGY 1281 are shown. (A) Changes in expression of Bad and Bid after incubation with *H. pylori*. (B) Induction of Bad and Bid by membrane fraction (MF), membrane fraction pretreated with proteinase K (MF-PK), cytosolic fraction (CF), concentrated supernatant (SP), or *E. coli* HB101 (HB101). Each fraction was added to AGS cells at a protein concentration of 10 μg/ml. *E. coli* HB101 was added at a bacterium/cell ratio of 10:1. Resuts at 24 h are shown as representative. (C) Effect of the caspase-8 inhibitor on expression of Bad and Bid after incubation with *H. pylori*. (D) Effect of the caspase-9 inhibitor on expression of Bad and Bid after incubation with *H. pylori*.

with previous studies implicating this caspase activity in the mitochondrial pathway.

Addition of serum to the medium, by which induction of apoptosis was significantly suppressed, as shown in Fig. 1C, had no apparent inhibitory effect on the expression of Bad and Bid (data not shown), indicating that the antiapoptotic effect of serum works on the downstream steps or on other signaling pathways.

DISCUSSION

In this study we demonstrate that *H. pylori* induces apoptosis through a pathway involving the sequential induction of caspase-8 activity, the proapoptotic proteins Bad and Bid, caspase-9 activity, and effector caspase-3 activity (Fig. 5). Although the mechanism by which caspase-8 induces Bad and Bid has been unclear until now, our results indicate that the activation of caspase-8 is an initiator for the downstream signaling cascade. This pathway appears to play a critical role in the early phase and induces a rather low degree of apoptosis. In the late phase, when a relatively high degree of apoptosis is induced, alternative pathways would likely mediate *H. pylori*-induced apoptosis. In both phases, contact of the bacterial membrane protein(s) with the host cell appears to trigger initiation of the apoptotic pathways, and apoptosis was significantly inhibited by the addition of serum. Serum appears to exert an antiapoptotic effect on both the host cell and the bacteria. These results indicate that the pathogenic activities of *H. pylori* would be largely affected by environmental conditions. We used AGS cells for these studies, since the AGS cell line serves as a suitable model for investigating these apoptotic pathways compared with other available gastric cell lines (13, 34). Since AGS cells undergo apoptosis rather than necrosis in response to the infection with *H. pylori*, it has been suggested that the response of AGS cells to infection with the bacterium mimics an in vivo setting (13, 34).

Recently, several bacterial pathogens have been found to trigger apoptosis in host cells in vitro or in vivo, and several types of mechanisms have been elucidated (27). Introduction of bacterial proteins into the host cell via the type III secretion pathway has been demonstrated to be involved in triggering apoptosis by enteropathogenic *E. coli* (17), *Salmonella* (52), *Shigella* (79, 80), and *Yersinia* (51). In enterohemorrhagic *E. coli*-mediated apoptosis, secreted Shiga toxins trigger apoptotic signals by binding to the receptor on the host cell (4, 35, 40, 41). However, *H. pylori*-induced triggering of cell death appears to differ from that resulting from the enteric bacteria, since exposure of the host cell to an *H. pylori* membrane protein(s) was sufficient to trigger the apoptotic pathways. In addition, compared to the enteric bacterium-mediated apoptosis, *H. pylori* is much weaker in its ability to cause cell death. The enteric bacteria cause more than 50% of cell death within a few hours. In contrast, *H. pylori* induced only up to 25% of host cell death in 24 h. In *Pseudomonas aeruginosa* (12) and *Campylobacter jejuni* (77), apoptotic activities of the outer membrane porins have been identified. Incubation of the porins purified from these organisms with the host cell causes a rather small degree of apoptosis (12, 77), which is comparable to that of *H. pylori*. In this mechanism, by interacting with the plasma membranes of the host cells, porins become embedded as hydrophilic pores in the phospholipid bilayer, damaging the structure and function of this part of the host cell architecture and leading to the activation of apoptotic signaling pathways (12). Since the *H. pylori* membrane fraction prepared as described in Materials and Methods contains a variety of membrane proteins, including outer membrane porins, involvement of this type of mechanism could be possible. Although *H. pylori* porins possess immunological activities, including the release of a series of inflammatory mediators (71), their apoptotic activities remain to be elucidated. Recently apoptosis-inducing activity of *H. pylori* urease was reported (24). *H. pylori* urease is suggested to be present on the surface of the bacterium (20). However, in our preliminary examination, the membrane fraction preparation that had no apoptosis-inducing activity

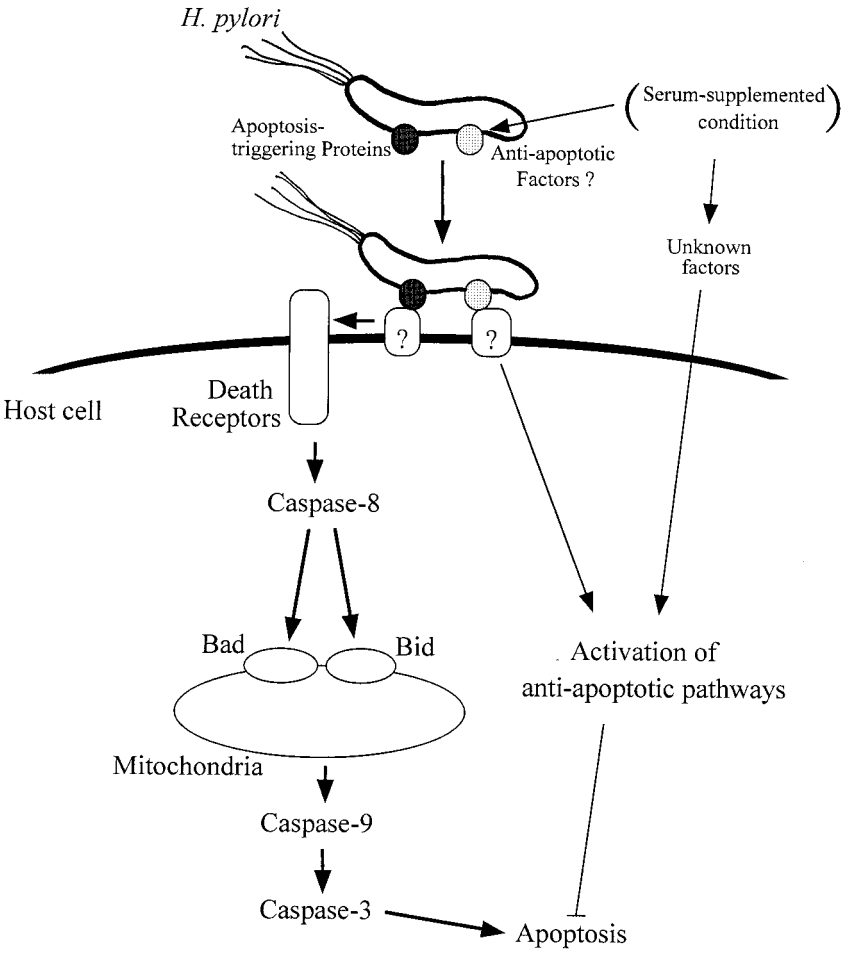

FIG. 5. Possible mechanism for the *H. pylori*-induced apoptotic signaling pathway. The apoptosis-triggering protein(s) attaches to the host cell surface and then stimulates the death receptors through unknown systems. Stimulation of the death receptors induces apoptosis through sequential induction of caspase-8 activity, proapoptotic proteins Bad and Bid, caspase-9 activity, and caspase-3 activity. Under the serum-supplemented condition, expression of antiapoptotic factors of *H. pylori* would presumably increase, leading to activation of antiapoptotic pathways of the host cell. Antiapoptotic substances, such as growth factors, would also be responsible for inhibition of *H. pylori*-mediated apoptosis.

did have apparent urease activity (data not shown), suggesting an involvement of another membrane protein(s) in triggering apoptosis.

Our results showed a potent antiapoptotic effect of serum on both AGS cells and the bacteria. Antiapoptotic substances, such as growth factors, would be responsible for the inhibition of apoptosis of the host cells. The mechanism by which serum eliminates the apoptotic activity of *H. pylori* is unclear. However, an antiapoptotic substance(s) expressed by *H. pylori* in response to serum would likely be involved, since *H. pylori* infection could stimulate potential antiapoptotic signals, including tyrosine kinases (2, 46, 58, 63–65, 68), protein kinase C (5, 6, 69), and the transcription factor NF-κB (25, 29, 32, 39, 48, 57). The ability of *H. pylori* to stimulate antiapoptotic pathways is not surprising, since there appears to be no obvious benefit for *H. pylori* from rapid host cell killing, which would result in the loss of colonization sites. The variability in the apoptosis-inducing activity of *H. pylori* might be a result of the adaptation of the bacterium to environmental conditions.

In the human stomach, the degree of apoptosis induced is affected by the associated inflammatory response. *H. pylori* infection induces a number of inflammatory mediators, including cytokines and chemokines (10). In vitro studies demonstrated that IFN-γ and tumor necrosis factor alpha, which are increased in the gastric mucosa during *H. pylori* infection (10, 38), augment the apoptosis induced by *H. pylori* (61, 72). IFN-γ is postulated to upregulate the expression of the Fas receptor on gastric epithelial cells (61, 73), and these tumor necrosis factor receptors activate caspase-8 (11, 50, 55). Taken together, our results support and extend recent evidence indicating the functional role of the tumor necrosis factor receptor family in *H. pylori*-induced apoptosis (31, 34, 61, 72, 73). The pathway initiated by the activation of caspase-8 that causes a rather small degree of apoptosis may be involved in the latent pathomorphisms in vivo. Activation of alternative pathways that induce a high degree of apoptosis may lead to severe cell loss, which is characteristic of ulceration. The environmental conditions in the stomach would exert a significant influence on the stimulation of these pathways. To further delineate the role of the pathways in the pathogenesis of *H. pylori*-mediated disease, an examination of the long-term time course of apoptosis and proliferation will be required (23), and in vivo stud-

ies, including those using animal models of human disease (44), should be undertaken.

To date, strain-specific genetic diversity has been proposed to be involved in the organism's ability to cause different diseases or even to be beneficial to the infected host and participate in the lifelong chronicity of infection (1, 3, 9, 47). However, our results suggest the need for further studies on host factors for a better understanding of the pathogenicity of *H. pylori*.

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