Analysis of apoptotic and antiapoptotic signalling pathways induced by Helicobacter pylori

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Background and aims: Although it is reported that Helicobacter pylori induces apoptosis on gastric epithelial cells, the mechanism remains unknown. Antiapoptotic effects generated by H pylori have not yet been evaluated.

Methods: (1) H pylori strains (type 1 wild, TN2-ΔcagE, TN2-ΔvacA) were cocultured with MKN45, TKM1, and HelA cells, and cell viability and apoptosis were assessed by trypan blue exclusion and DNA laddering, respectively. (2) Activation of caspases-3, 7, and 8, cytochrome c release from the mitochondria, and Fas, Fas associated death domain protein (FADD), Bax, Bak, and Bcl-X expression were evaluated by immuno blot analysis. (3) To investigate whether nuclear factor kappa B (NFκB) activation induced by cag pathogenicity island (PAI) positive H pylori affects antiapoptosis, MKN45 cells stably expressing super-repressor IkBα were cocultured with H pylori, and cell viability and caspase activation were evaluated. NFκB regulated gene expression was also evaluated by ribonuclease protection assay.

Results: (1) Wild-type and ΔvacA mutant H pylori induced apoptosis more potently than the ΔcagE mutant. Inhibition of cell contact between H pylori and cancer cells and heat killing H pylori diminished cell death. (2) Caspases-3, 7, and 8 were activated time dependently by H pylori as well as by the agonist anti-Fas. Cytochrome c release from mitochondria was observed and was not inhibited by caspase-8 inhibitor. Although protein expression of Fas, FADD, Bax, Bak, and Bcl-X in the whole cell lysates was not changed by H pylori, Bax was decreased from mitochondria free cytosol suggesting that Bax was translocated into mitochondria. (3) Cell death and the activities of caspases-3 and 8 were promoted in MKN45 cells stably expressing super-repressor IkBα that inhibits NFκB activation. Antiapoptotic proteins c-IAP1 and c-IAP2 were upregulated by the wild-type strains.

Conclusion: cag PAI positive H pylori is capable of inducing apoptotic effects mainly through the mitochondrial pathway. Antiapoptotic effects mediated by NFκB activation were also observed.

Abbreviations: PAI, pathogenicity island; NFκB, nuclear factor kappa B; FADD, Fas associated death domain protein; IFN, interferon; PBS, phosphate buffered saline; BSA, bovine serum albumin; VacA, vacuolating cytotoxin; FBS, fetal bovine serum; MyD88, myeloid differentiation factor 88.
responsible for transcription factor nuclear factor kappa B (NFκB) activation.\(^{2,16}\) Isogenic mutant studies demonstrated that some proteins encoded by cag PAI genes are responsible for NFκB activation.\(^{17}\) NFκB is a regulator of genes involved in inflammation, cell proliferation, and apoptosis.\(^{24,25}\) Recent studies suggest that NFκB may play a critical role in protecting cells against apoptosis.\(^{26,27}\) The antiapoptotic role played by NFκB involves the activation of this transcription factor to induce expression of genes that promote cell survival such as the genes coding for TRAF1, TRAF2, and the cellular inhibitors of apoptosis 1 and 2 (c-IAP1, c-IAP2).\(^{28}\) Curiously, NFκB has been found to be associated with proapoptotic as well as antiapoptotic mechanisms. For instance, NFκB activation appears to induce apoptosis in cells exposed to hydrogen peroxide.\(^{29}\) The magnitude of the stimulus and the cell type involved may determine whether NFκB leads to cell survival or cell death.

Although \textit{H pylori} infection induces apoptosis in gastric epithelial cells, the mechanism of intracellular signal conduction that leads to apoptosis is scarcely known. In addition, it is not known whether \textit{H pylori} mediated NFκB activation plays an apoptotic or antiapoptotic role. The aims of this study were to clarify the molecular mechanism of the proapoptotic pathway induced by \textit{H pylori}, and to investigate the relation between \textit{H pylori} induced NFκB activation and apoptosis.

**MATERIALS AND METHODS**

**Bacterial strains**

TN2, a strain positive for CagA, cag PAI, and VacA (vacuolating cytoxin), were generously provided by Dr Nakao (Takeda Chemical Industries Ltd, Osaka, Japan). Infection with this strain induces gastric cancer in Mongolian gerbils.\(^{30}\) Isogenic \textit{cagE} negative and \textit{vacA} negative mutant strains, TN2-\textit{ΔcagE}, and TN2-\textit{ΔvacA} were prepared by insertion of a kanamycin resistant gene into the \textit{cagE} and \textit{vacA} locus of TN2, as previously described.\(^{31}\) \textit{H pylori} strains were cultured on Columbia agar with 5\% (vol/vol) horse blood and Dent antibiotic supplement (Oxoid, Basingstoke, UK) at 37°C for three days under microaerobic conditions (Campy-Pak Systems; BBL, Cockeysville, Maryland, USA). The isolates were kept at ~80°C in Brucella broth with 5\% (vol/vol) fetal bovine serum (FBS) containing 16\% (vol/vol) glycerol. In coculture experiments, \textit{H pylori} was cultured in Brucella broth containing 7.5\% FBS for 24 hours, centrifuged, and resuspended in cell culture medium (RPMI 1640) containing 10\% FBS, and then applied immediately to assays. The range of bacteria: cancer cell ratio was 50:1 to 75:1 when cocultured with 10\(^4\) colony forming units/ml. Heat killed \textit{H pylori} bodies were prepared by heating at 80°C for 30 minutes.

**Plasmids and reagents**

The super-repressor mutant of \textit{IκB}α, \textit{IκB}α (SS32/36AA) subcloned in pcDNA3, was generously donated by Dr Suzuki (Yamanouchi Pharmaceutical Co, Ltd, Ibaraki, Japan).\(^{32}\) The anti-FLAG monoclonal antibody M2 antibody was purchased from Sigma (St Louis, Missouri, USA); the polyclonal anti-Fas antibody, polyclonal anti-I\(\alpha\)B\(\kappa\) from Sigma (St Louis, Missouri, USA); the polyclonal anti-cytochrome c antibody from Peptide Institute, Inc (Osaka, Japan); and recombinant human interferon \(\gamma\) (IFN-\(\gamma\)) from Pepro Tech EC (London, UK).

**Human cell lines**

Human gastric cancer cells MKN45 and TM1K were maintained in RPMI-1640 containing 10\% FBS, l-glutamine, 100 U of penicillin-G, and 100 µg/ml of streptomycin. MKN45 was obtained from the Riken Gene Bank (Tsukuba, Japan). TM1K was provided by Dr E Tahara (Hiroshima University, Hiroshima, Japan). HeLa cells were maintained in Dulbecco’s modified Eagle’s medium containing 10\% FBS, l-glutamine, 100 U of penicillin-G, and 100 µg/ml of streptomycin. HeLa cells were obtained from the Riken Gene Bank.

**Cell viability assay**

Cells were collected and washed in phosphate buffered saline (PBS). Cell viability was assessed by trypan blue dye exclusion assay by counting 300 cells. Viable cells were quantitated under bright field microscopy.

**Analysis of DNA fragmentation**

Cancer cells cocultured with \textit{H pylori} or treated with anti-Fas (CH-11) (approximately 1x10\(^4\)cells) were washed in PBS and lysed in 40 µl of lysis buffer containing 200 mM NaHPO\(_4\), and 4 mM citric acid. Samples were centrifuged and the supernatants were incubated with 3 µl of 0.25% NP-40 and 3 µl of Dnase-free RNase (10 mg/ml), and followed with a 10 mg/ml proteinase K digestion. Aliquots (10 µl) from a 50 µl DNA solution were electrophoresed on a 2% agarose gel.

**Immunoblot analysis**

Cells were suspended in 50 mM Tris HCl (pH 7.4) buffer containing 1 mM EGTA, 2 mM DTT, 25 mM sodium β-glycerophosphate, 0.1 mM PMSE, and 10 µg/ml aprotinin. An equal amount of protein extracts was fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and electrophotochemically transferred to a PVDF membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was probed with the antibodies described above. An ECL detection assay (Amersham Pharmacia Biotech) was performed according to the manufacturer’s instructions.

**Immunoprecipitation**

MKN45 cells (5x10\(^4\)) were incubated with \textit{H pylori} or the agonist anti-Fas (CH-11) for 12 hours, and lysed in 50 mM Tris-HCl (pH 7.4) buffer containing 1 mM EGTA, 2 mM DTT, 25 mM sodium β-glycerophosphate, 0.1 mM PMSE, and 10 µg/ml aprotinin. Lysates were immunoprecipitated with anti-Fas antibody (Santa Cruz) and 20 µg of protein A-Sepharose. Immunoprecipitates were washed five times in PBS and used for immunoblot analysis.

**Generation of stable transfectants**

MKN45 (5x10\(^4\)) were seeded onto 10 cm plates and transfected 24 hours later with 3 µg of FLAG-I\(\kappa\)Bα (SS32/36AA) subcloned in pcDNA3 or control vector pcDNA3 using Effectene transfection reagent (Quiagen, Hilden, Germany). Cells stably expressing FLAG-I\(\kappa\)Bα (SS32/36AA) were selected in medium containing 1 µg/ml G418 (Gibco BRL, Life Technologies, Inc., Rockville, Maryland, USA) for two weeks. MKN45 cells transfected with the pcDNA3 vector and selected by G418 were used as controls.

**RNA isolation and nuclelease protection assay**

Total RNA was extracted using an acid guanidinium thiocyanate-phenol-chloroform method according to the manufacturer’s instructions (Isogen; Nippongene, Tokyo, Japan). Total RNA (10 µg) was hybridised with antisense RNA probes labelled with \(32\)P-UTP using the apoptosis related template set, hAPO-2 (bcl-X L/S, bcl1, bik, bax, bcl-2, mcl-1, and...
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Electrophoretic mobility shift assay

Absorbance was measured at 405 nm. 12 hours and lysed, and supernatants were used for ELISA.

DNA binding was considered significant.

Statistics

A Fujix bioimaging analyser FLA 3000 (Fuji Photo Film). The gel was dried and autoradiography was performed using a Fuji X-system (Fuji Photo Film Co, Ltd, Tokyo, Japan).

DNA fragmentation ELISA

DNA fragmentation was quantified using a commercially available ELISA (Boehringer Mannheim Biochemicals, Mannheim, Germany) that detects nucleosidic fragments in the cytoplasmatic fraction of cells undergoing apoptosis but not necrosis. For these experiments, 5x10^6 cells were incubated in triplicate with H pylori, anti-Fas (CH-11), or medium alone for 12 hours and lysed, and supernatants were used for ELISA. Absorbance was measured at 405 nm.

Electrophoretic mobility shift assay

Detection of NFkB was performed with a 32P dATP labelled oligo probe containing the NFkB recognition site purchased from Promega (Madison, Wisconsin, USA). The DNA binding reactions were performed at room temperature for 30 minutes in a 10 μl mixture consisting of 4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 50 mM NaCl, and 0.5 μg of poly(dI-dC). Supershift analysis was performed using antibodies against p65 and p50. DNA-protein complexes were loaded onto a chilled 4% non-denaturing acrylamide gel. Gel electrophoresis was executed in 0.5×Tris borate-EDTA at 4°C. The gel was dried and autoradiography was performed using a Fujix image analyser FLA3000 (Fuji Photo Film).

Effects of H pylori virulence factors

To evaluate the effects of H pylori virulence factors, the cagE mutant TN2-ΔcagE and the vacA mutant TN2-ΔvacA were used. In the cell viability assay, wild-type and TN2-ΔvacA significantly decreased the viability of MKN45 and TMK-1 cells after 24 and 36 hours of coculture (fig 2A). The DNA fragmentation assay revealed that wild-type and TN2-ΔvacA induced apoptosis after 16 hours of infection whereas TN2-ΔcagE did not induce apoptosis at this time (fig 2B). However, cagE also induced apoptosis after 36 hours of infection (data not shown). These results indicate that the cagE mutant induces apoptosis less effectively than the wild-type or vacA mutant.

Effects of heat killed H pylori and inhibition of direct contact with host cells

To assess the effect of direct contact, cancer cells and bacteria were separated by a membrane filter (Nunc Tissue Culture Inserts No 162138; Nunc, Roskilde, Denmark). We also used heat killed bacteria heated at 80°C for 30 minutes. These procedures suppressed the H pylori mediated decrease in cell viability (fig 3). No DNA fragmentation was observed with heat killed bacteria or with viable bacteria separated by a permeable membrane, indicating that direct contact with viable bacteria is necessary for induction of apoptosis by H pylori.

Activation of caspases-8, 3, and 7

To assess whether H pylori induces apoptosis via caspase activation, immunoblot analysis was performed using anti-caspase-8, 3, 7, and cleaved caspase-3 antibodies in MKN45
cells. In untreated MKN45 cells, caspase-8 was present primarily as a molecule of approximately 32 kDa. Incubation with H pylori or anti-Fas resulted in a time dependent degradation of the primary form that was processed to a 20 kDa active form. The active form of caspase-3 was observed 12 hours after H pylori treatment. In untreated MKN45 cells, caspase-7 was present primarily as a molecule of approximately 35 kDa. Incubation with H pylori and anti-Fas resulted in a time dependent degradation of the primary form. An increase in the processing of caspase-7 was observed 12 hours after H pylori treatment. These results indicate that H pylori and anti-Fas activated caspases-8, 3, and 7 in MKN 45 cells (fig 4). Similar results were obtained using TMK-1 cells (data not shown).

**Caspase-8 and Fas do not play a major role in H pylori induced apoptosis**

We evaluated the effect of the caspase-8 inhibitor Ac-IETD-CHO on H pylori mediated apoptosis in MKN45 cells. While the inhibitor (100 µM) abolished anti-Fas induced cell death (from 50% to 15%), H pylori mediated cell death was not affected (fig 5A), and DNA fragmentation induced by H pylori was not inhibited (fig 5B). These results indicate that caspase-8 activation by H pylori does not function as a major pathway in H pylori induced apoptosis. Similar results were obtained using TMK-1 cells (data not shown).

We evaluated whether FADD was recruited to Fas and found that under apoptosis induced conditions, anti-Fas but not H pylori recruited FADD to Fas (fig 6A). In evaluating the effect of neutralising anti-Fas antibody (ZB-4), we found that ZB-4 decreased cell cytotoxicity induced by the agonist anti-Fas antibody (CH-11) (from 42% to 21%) but not H pylori mediated cell cytotoxicity (from 35% to 38 %) (fig 6B). These results suggest that Fas does not play a major role in H pylori induced apoptosis.

**H pylori induces cytochrome c release from the mitochondria**

To examine whether or not H pylori induced apoptotic signaling involves a mitochondrial pathway, MKN45 cells were treated with H pylori, and mitochondria free cytosolic extracts were prepared and analysed by immunoblotting. Cytochrome c accumulated in cytosolic extracts after 12 hours of coculturing with H pylori. In examining the effects of the agonist anti-Fas antibody, as previously reported in the other cell lines, cytochrome c also accumulated after exposure to anti-Fas in MKN45 cells (fig 7A). Similar results were obtained using TMK-1 cells. To examine whether or not cytochrome c accumulation is caspase-8 dependent, cells were pretreated with caspase-8 inhibitor (Ac-IETD-CHO) for one hour before exposure to H pylori or anti-Fas. Although anti-Fas mediated...
cytochrome c release from the mitochondria was inhibited by the caspase-8 inhibitor, \textit{H pylori} mediated release was not inhibited. We also used the pan-caspase inhibitor Z-VAD-FMK. Similar to the caspase-8 inhibitor, the pan-caspase inhibitor abolished anti-Fas mediated cytochrome c release from the mitochondria but not \textit{H pylori} mediated release. This indicates that \textit{H pylori} induced cytochrome c release from the mitochondria is not caspase-8 dependent (fig 7B).

**Expression of apoptosis related proteins**

We determined whether \textit{H pylori} mediated apoptosis correlated with expression of apoptosis related proteins. Coculture with \textit{H pylori} cells did not change levels of Fas, FADD, Bax, Bak, or Bcl-X in total cell lysates extracted from MKN45 at the indicated times (fig 8A). Similar results were obtained using TMK-1 cells. We also evaluated mRNA expression of Bax, Bak, and Bcl-X using a ribonuclease protection assay and found no apparent changes. It is known that Bax may be translocated from the cytosol to the mitochondrial membrane without changing total cell amount. Thus we examined the immunoblot analysis of Bax using only the cytosolic fraction and found that cytosolic levels of Bax were decreased in \textit{H pylori} mediated apoptosis as well as in Fas mediated apoptosis, indicating that Bax is translocated into mitochondria in \textit{H pylori} mediated apoptosis (fig 8C). We also evaluated the effect of the caspase-8 inhibitor. The inhibitor abolished the decrease in cytosolic Bax induced by anti-Fas but did not affect the decrease induced by \textit{H pylori} (fig 8C).

**\textit{H pylori} mediated NF\textsubscript{KB} activation suppresses apoptotic effects**

We previously described \textit{cag} PAI positive \textit{H pylori} activation of NF\textsubscript{KB} in gastric cancer cells. There was a significant decrease in induction of NF\textsubscript{KB} transiently transfected with mutant IkB\textalpha (S32A/S36A) (super-repressor), indicating that phosphorylation of IkB\textalpha at Ser32 and Ser36 is critical for \textit{H pylori} mediated activation of NF\textsubscript{KB}. To determine whether NF\textsubscript{KB} activation correlated with \textit{H pylori} induced apoptosis, we generated stable transfectants of MKN45 cells expressing mutant IkB\textalpha (MKN45 IkB\textalpha SR) (fig 9A). To examine the effect of stable expression of IkB\textalpha super-repressor, we performed a electrophoretic mobility shift assay. \textit{H pylori} and tumour necrosis factor \textalpha activated NF\textsubscript{KB} in MKN45-IkB\textalpha SR cells significantly less than in wild-type MKN45 cells. This result indicated that stable expression of IkB\textalpha super-repressor construct is functioning as an inhibitor (fig 9B). Cell cytotoxicity caused by \textit{H pylori} or the agonist anti-Fas was evaluated in the IkB\textalpha (S32A/S36A) transfectants and compared with control cells. Both \textit{H pylori} and the agonist anti-Fas significantly decreased the viability of the transformant cells at 12,
apoptosis was increased in MKN45 I cells compared with MKN45 cells, indicating that specific activated more rapidly (fig 9E). These results indicate that Caspase-8 and caspase-3, as revealed by immunoblot, were the mitochondria is a major pathway in DISCUSSION

In this study we have shown that cytochrome c release from the mitochondria is a major pathway in H pylori mediated apoptosis. Although caspase-8 was indeed activated, the caspase-8 inhibitors did not affect H pylori mediated apoptosis, indicating that the major pathway is independent of caspase-8 activation (fig 10).

Activation of caspase-8 usually occurs as a consequence of surface receptor binding to its ligand. Reportedly, the Fas-Fas ligand system is involved in H pylori mediated apoptosis in vivo and in vitro but in this study neutralising anti-Fas antibody did not affect H pylori mediated apoptosis in cultured cancer cells. Moreover, we found no evidence of recruitment of FADD to Fas, a downstream event of Fas-Fas ligand association, in H pylori mediated apoptosis. Thus involvement of the Fas-Fas ligand system in H pylori mediated apoptosis was not substantiated, and the mechanism of caspase-8 activation has yet to be clarified. In addition, the caspase-8 inhibitor Ac-IETD-CHO did not inhibit H pylori mediated apoptosis, suggesting that caspase-8 dependent signal transduction does not play a major role in H pylori mediated apoptosis, at least in vitro. Although it is reported that the Fas-Fas ligand system is important in vivo, activation of this pathway may be induced indirectly and secondary. We suggest that H pylori is capable of inducing apoptosis mainly through the mitochondrial pathway in vitro. The importance of this pathway in vivo remains unknown.

Various proapoptotic signals, such as those induced by radiation, anticancer drugs, and stress, converge at cytochrome c release from the mitochondria. Although the current data suggest that cytochrome c release also plays a pivotal role in H pylori mediated apoptosis, the exact mechanism has yet to be investigated. The status of Bcl-2 family proteins determines whether a cell will live or die through regulation of cytochrome c release from the mitochondria. Increased Bak expression, a proapoptotic member of the Bcl-2 family, in H pylori infection was recently reported. However, we did not find changes in expression of Bax, Bak, or Bcl-X in either mRNA or protein levels when cocultured with H pylori. These findings do not exclude the possibility that some Bcl-2 family proteins are involved in H pylori mediated apoptosis. For example, Bax may translocate from the cytosol to the mitochondria for integration into the membrane following a proapoptotic stimulus. This action then results in cytochrome c release while the total amount of protein remains constant. Thus we checked by immunoblot analysis the level of Bax in the cytosolic fraction and found that cytosolic Bax was decreased in H pylori mediated apoptosis as well as in Fas mediated apoptosis, suggesting that Bax was translocated into mitochondria in H pylori mediated apoptosis.

p53 is an important molecule that affects apoptosis. Bax gene expression caused by p53 may be an important part of p53 mediated apoptosis. Reportedly, H pylori infection in patients resulted in nuclear staining for p53 in the glandular cells of the mucosa, and bacterial eradication caused a decrease in p53 accumulation in epithelial cells. However, we did not find upregulation of Bax or MDM2, which are regulated by p53 in cells with normal p53, including MKN45 cells, suggesting that H pylori cannot activate p53 directly in vitro (unpublished observation).

We have previously shown that H pylori induces NFκB activation. In this study, we confirmed that H pylori mediated NFκB activation exerts antiapoptotic effects in MKN45 cells where upregulation of c-IAP1 and 2 may be involved. Thus H pylori has both apoptotic and antiapoptotic effects. Similarly, the cytokine tumour necrosis factor α can produce bidirectional effects on apoptosis. The signal triggered by tumour necrosis factor α binding to its receptor bifurcates at TRADD: one signal induces NFκB activation via RIP promoting cell survival and the other induces apoptosis via FADD. The adapter molecule myeloid differentiation factor 88 (MyD88) mediates both apoptosis and NFκB activation through Toll-like receptors. Inhibition of the NFκB pathway downstream of MyD88 potentiates apoptosis, indicating that these two pathways bifurcate at the level of MyD88. Moreover, MyD88 binds FADD and is sufficient to induce apoptosis. Recently, we revealed that NFκB activation caused by H pylori was associated with TRAF6, a molecule downstream of MyD88. In addition, as H pylori is a gram negative bacterium, the relation to Toll-like receptors should be investigated.

Knockout of cagE, one of the cag PAI genes, decreased both the apoptotic effects and potential to activate NFκB. Thus it

24, and 36 hours (fig 9C). To compare levels of DNA fragmentation, we used fragmentation ELISA at 12 hours after infection. DNA fragmentation was increased in MKN45 1xBtR SR cells compared with MKN45 cells, indicating that specific apoptosis was increased in MKN45 1xBtR SR cells (fig 9D). Caspase-8 and caspase-3, as revealed by immunoblot, were activated more rapidly (fig 9E). These results indicate that NFκB activation by H pylori demonstrated antiapoptotic effects. To evaluate expression of apoptosis related genes, we performed a ribonuclease protection assay. C-IAP1 and IAP2 were upregulated by H pylori infection in MKN45 cells. cagE mutant H pylori demonstrated a smaller potential to upregulate these genes (fig 9F). These results indicate that H pylori may exert antiapoptotic effects by upregulating c-IAP genes through NFκB activation.
were prepared from MKN45 and MKN45 I sequences (electrophoretic mobility shift assay). The nuclear extracts and total RNA was extracted at the indicated times. The ribonuclease protection assay was performed according to the protocol.

Figure 9

(A) Detection of stable MKN45 transfectants expressing IκBα (SS32/36AA). The clone was analysed using a monoclonal antibody to FLAG and polyclonal antibody to IκBα. (B) Specific protein binding activities of nuclear factor kappa B (NFκB) sequences (electrophoretic mobility shift assay). The nuclear extracts were prepared from MKN45 and MKN45 IκBα SR cells. Cells were treated or not treated with Helicobacter pylori (HP) or 10 ng/ml tumour necrosis factor α (TNF-α) for 90 minutes. Nuclear extracts was incubated with 32P labelled oligonucleotide for 30 minutes. (C) MKN45 and MKN45 IκBα SR cells were treated with interferon γ (IFN-γ) 10 ng/ml for 24 hours. Cells were incubated with H pylori (HP) or anti-Fas (CH-11) for 24 hours. Cell viability was assessed by trypan blue dye exclusion assay by counting 300 cells. Viable cells were quantitated under bright field microscopy. Results are expressed as percentages of dead cells. Values are mean (SD) of three independent experiments. *Percentage cytotoxicity was significantly (p<0.05) different between the MKN45 treated cells with anti-Fas or cocultured with H pylori and MKN45 IκBα SR treated cells with anti-Fas or cocultured with H pylori. (D) DNA fragmentation was quantified using a commercially available ELISA (Boehringer Mannheim Biochemicals, Mannheim, Germany); 5×105 cells were incubated in triplicate with H pylori (HP), anti-Fas (CH-11), or medium alone for 12 hours and lysed, and the supernatants were used for ELISA. Absorbance was measured at 405 nm. *Absorbance was significantly (p<0.05) different between the control and treated with anti-Fas or cocultured with H pylori in the MKN45 IκBα SR groups; NS, no significant difference was found. (E) MKN45 and MKN45 IκBα SR cells were treated with IFN-γ (10 ng/ml) for 24 hours. Cells were incubated with H pylori (HP) or anti-Fas (CH-11) for 12 hours. Immunoblot analysis was performed using anti-caspase-8, caspase-3, cleaved caspase-3, and anti-actin antibody. (F) MKN45 and THP-1 cells were incubated with H pylori and total RNA was extracted at the indicated times. The ribonuclease protection assay was performed according to the supplier’s instructions.

Figure 10

Schematic representation of the signalling pathways leading to apoptosis and antiapoptosis in response to Helicobacter pylori in gastric epithelial cells.

may be speculated that both apoptosis and NFκB activation are triggered by the same signal that is dependent on cag PAI. Several cag PAI proteins, including CagE, are thought to constitute a type IV secretion system. CagE itself is a homologue of a transporter component in Agrobacterium tumefaciens and Bordetella pertussis that engage in the transcellular transport of toxins or T-DNA. Recent reports suggest that the system transports CagA protein, another product of the cag PAI, into the cytoplasm of host cells, where CagA undergoes tyrosine phosphorylation. As the system may also transport other molecules, it is possible that a certain protein produced by H pylori is transported into host cells where it triggers both apoptotic and NFκB activating signalling pathways. Recently, we have revealed that knockout of the cagE gene deprived wild-type H pylori on the pathogenicity for gastric ulcer, gastritis, and intestinal metaplasia in an in vivo model. This observation may indicate that the pro- and antiapoptotic effect induced by wild-type H pylori is an important factor in the pathogenesis of H pylori mediated gastric diseases.

In conclusion, we demonstrated that H pylori directly induced apoptosis mainly through cytochrome c release from the mitochondria. H pylori also exerted antiapoptotic effects through NFκB activation. Both apoptotic and antiapoptotic effects were dependent on cag PAI.

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