Helicobacter pylori induces Prostaglandin E₂ Synthesis Involves Activation of Cytosolic Phospholipase A₂ in Epithelial Cells

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Helicobacter pylori initiates an inflammatory response and gastric diseases, which are more common in patients infected with H. pylori strains carrying the pathogenicity island, by colonizing the gastric epithelium. In the present study we investigated the mechanism of prostaglandin E₂ (PGE₂) synthesis in response to H. pylori infection. We demonstrate that H. pylori induces the synthesis of PGE₂ via release of arachidonic acid predominately from phosphatidylglycerol. In contrast to H. pylori wild type, an isogenic H. pylori strain with a mutation in the pathogenicity island exerts only weak arachidonic acid and PGE₂ synthesis. The H. pylori-induced arachidonic acid release was abolished by phospholipase A₂ (PLA₂) inhibitors and by pertussis toxin (affects the activity of Goα/Goβ). The role of phospholipase C, diacylglycerol lipase, or phospholipase D was excluded by using specific inhibitors. An inhibitor of the stress-activated p38 kinase (SB202190), but neither inhibitors of protein kinase C nor an inhibitor of the extracellular-regulated kinase pathway (PD98059), decreased the H. pylori-induced arachidonic acid release. H. pylori-induced phosphorylation of p38 kinase and cytosolic PLA₂ was blocked by SB202190. These results indicate that H. pylori induces the release of PGE₂ from epithelial cells by cytosolic PLA₂ activation via Goα/Goβ proteins and the p38 kinase pathway.

The Helicobacter pylori infection induces the release of a number of proinflammatory cytokines and chemokines from the gastric epithelium (1) and plays a critical role in the development of gastritis, peptic ulcer disease, and rarely, in gastric carcinoma (2, 3). Evidence has been presented that an increase of prostaglandins (PGs) in gastric tissue from patients may play a crucial role in H. pylori infection (4). In gastrointestinal epithelia, PGE₂ is implicated in maintaining the normal function and structure of the gastric mucosa by modulating diverse cellular functions such as secretion of fluid and electrolytes, mucosal blood flow, and cell proliferation (5, 6).

Studies have shown that H. pylori strains differ in their virulence and in their ability to trigger the induction of inflammatory mediators in gastric epithelial cell lines (1). The response is more intense to strains carrying the cagA gene. The analysis of the genomic region containing the cagA gene revealed a 40-kilobase DNA region, which represents a pathogenicity island (PAI) and codes for 31 genes (7). Upon contact with the gastric epithelium, PAI-encoded components contribute in a specialized type IV secretion machinery that translocates the CagA protein into the eukaryotic target cell where it is phosphorylated on tyrosine residues (8–12). H. pylori infection triggers by unknown bacterial factors multiple biochemical pathways in host cells including activation of transcription factors NF-κB and AP-1 (13–17), phospholipase C (PLC) (18), and the increase of the cytosolic free calcium concentration as well as the generation of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate (19). The activation of the PGE₂ signaling pathway in H. pylori-colonized gastric cells has not been studied so far.

One of the mechanisms for increased PG production in response to H. pylori infection is an induction of COX-2 expression (20, 21). Another rate-limiting step in the control of PG production is the release of AA from membrane phospholipids, which is known to occur via a number of different pathways. One involves the activation of phospholipase A₂ (PLA₂), others involve the action of PLC or phospholipase D (PLD) (22–24). In this report, we studied the control of PGE₂ and AA production in response to H. pylori infection of epithelial cells after specific labeling of potential phospholipid precursors and selective inhibition of enzymes involved in the pathways of AA production. The presented results provide evidence that colonization of epithelial cells by H. pylori induces a release of PGE₂ and AA by activation of the cytosolic PLA₂ (cPLA₂) via pertussis toxin-sensitive heterotrimeric Goα/Goβ proteins and the p38 stress-activated kinase cascade. This process does not seem to involve PLC or PLD pathways.

EXPERIMENTAL PROCEDURES

Bacteria—The isogenic H. pylori strains P12 wild type, cagA (mutation affect cagA with a probable polar effect in the PAI), and vacA (25)

SB202190, 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl) imidazole; SM, sphingomyelin; PTX, pertussis toxin.

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The abbreviations used are: PG, prostaglandin; PAI, pathogenicity island; AA, arachidonic acid; BIM, bisindolylmaleimide I; DAG, diacylglycerol; HELSS, halogen lactone suicide substrate; MAPF, methyl arachidonoylfluorophosphate; MOI, multiplicity of infection; PA, phosphatidic acid; PC, phosphatidylycholine; PD98059, 2-(2-amino-3-methoxyphenyl)-oxanaphthalen-4-one; PE, phosphatidylethanolamine; PET, phosphatidylethanol; PI, phosphatidylinositol; PLA₂, phospholipase A₂; iPLA₂, Ca²⁺-independent phospholipase A₂; cPLA₂, cytosolic phospholipase A₂; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylinerine;
were used for colonization of epithelial cell line cells. For cultivation, the bacteria were resuspended in brain heart infusion (Difco) medium. 10^5 bacteria were seeded on agar plates containing 10% horse serum and cultured for 48–72 h at 37 °C in a microaerophilic atmosphere (generated by Campy Gen, Oxoid, Basingstoke, UK). For stock culture, the bacteria were resuspended in brain heart infusion broth supplemented with 10% fetal calf serum (FCS, Life Technologies, Inc.) and 20% glycerol, and maintained at ~70 °C.

**Cell Culture and H. pylori Infection—** Gastric epithelial cells (AGS) and HeLa cells were grown in RPMI 1640 (Life Technologies, Inc.) supplemented with 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (Biochrom KG, Berlin, Germany), 20 mM L-glutamate (Sigma), and 10% FCS, in a humified atmosphere of 95% air and 5% CO2, at 37 °C.

**Determination of [3H]Inositol Phosphates—** For analysis of the formation of inositol phosphates, cells in 6-well plates were incubated for 24 h in 1 ml of RPMI 1640 medium containing 0.1% FCS and 2 μCi of [3H]inositol (ICN Biomedicals, Eschwege, Germany). Cells were washed twice with Hanks’ balanced salt solution and preincubated with 10 μM LiCl in this solution for 10 min at 37 °C. In some experiments, PLC inhibitor was added to the cells during this incubation.

**Lipid Analysis—** After the incubations, the medium was carefully removed for analysis, and lipids were extracted from the cells by the addition of chloroform, methanol, 20 mM acetic acid (50:220:10, v/v) to the dishes. In the case of the incubation with the media, chloroform (1:2.2, v/v) was added to give a single phase. The phase were split according to the method of Bligh and Dyer (26). The chloroform phase was dried, and lipids were applied on high performance thin layer chromatography (TLC, Merck) plates. For determination of lysoP1, the cells and media were extracted twice with chloroform/methanol (1:2, v/v). The organic extracts were dried and then subjected to butanol/ water (1:1, v/v). The radioabeled lipids were recovered in the butanol phases, which were washed with water, dried, and analyzed by TLC.

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**RESULTS**

*H. pylori Increases AA Release from Epithelial Cells—* To investigate whether *H. pylori* might elicit release of AA for PGE2 formation (PGE2 release was induced in HeLa and AGS cells infected with *H. pylori* wild type and an isogenic vacA mutant but not by an isogenic cagA mutant (data not shown)), [3H]labeled AA-labeled AGS and HeLa cells were incubated for different periods of time in the absence or presence of different *H. pylori* strains. To trap the released radioactivity (free AA and its metabolites) in the extracellular medium, incubation was performed in the presence of 0.1% bovine serum albumin.

Lipids were extracted from cells and their medium and analyzed by two-dimensional chromatography. As shown in Fig. 1, colonization of AGS (Fig. 1A) and HeLa cells (Fig. 1B) by the *P.2* wild-type strain increased the intracellular accumulation and extracellular release of free radiolabeled AA as compared with controls. Typically, a 3–6-fold increase of free AA and its metabolites in the extracellular medium, incubation was performed in the presence of 0.1% bovine serum albumin. Lipids were extracted from cells and their medium and analyzed by two-dimensional chromatography. As shown in Fig. 1, colonization of AGS (Fig. 1A) and HeLa cells (Fig. 1B) by the *P.2* wild-type strain increased the intracellular accumulation and extracellular release of free radiolabeled AA as compared with controls. Typically, a 3–6-fold increase of free AA and its metabolites in the extracellular medium, incubation was performed in the presence of 0.1% bovine serum albumin. Lipids were extracted from cells and their medium and analyzed by two-dimensional chromatography. As shown in Fig. 1, colonization of AGS (Fig. 1A) and HeLa cells (Fig. 1B) by the *P.2* wild-type strain increased the intracellular accumulation and extracellular release of free radiolabeled AA as compared with controls. Typically, a 3–6-fold increase of free AA and its metabolites in the extracellular medium, incubation was performed in the presence of 0.1% bovine serum albumin.
magnitude of the decrease ranged from 30 to 50% within the experiments. In terms of total counts, the amount of 14C-labeled AA that disappeared from the phospholipid pool in \( H. pylori \)-colonized cells matched approximately the amount of radioactivity released. Two-dimensional phospholipid analysis, which separates PS from PI, revealed that PI was the exclusive source of AA in the PS/PI spot. The percentage of \([14C]\)phosphatidylcholine (PC) decreased slightly, although these changes were only significant after longer time points (12% after 180 min). At longer time points, \( H. pylori \) caused also a small increase (14% after 180 min) in the percentage of \([14C]\)phosphatidylethanolamine (PE) presumably by interconversion of intact phospholipids and reincorporation of released AA into PE specifically. In melittin-treated cells (positive control), the phospholipids whose radiolabeled content decreased were PC and PI. Exposure of cells to \( P12 \) wild-type strain or treatment with melittin resulted also in a small but significant increase in \([14C]\)-labeled AA-labeled PA and probably reflects the production of diacylglycerol (DAG) via activation of PLC (see below) and its phosphorylation by DAG kinase (31).

Involvement of Cytosolic Phospholipase A\(_2\) in \( H. pylori \)-induced AA Release—As shown in Fig. 2, \( H. pylori \) (\( P12 \))-induced release of \([14C]\)-labeled AA was significantly inhibited by both mepacrine (30 and 100 \( \mu \)M), a nonspecific inhibitor of the PL\( A_2 \) (32), and MAFP (10 and 50 \( \mu \)M), a potent inhibitor of cPLA\(_2\) and Ca\(^{2+}\)-independent PL\( A_2 \) (iPLA\(_2\)) (33). In parallel with inhibition of AA release, both inhibitors abolished the decrease in the radiolabeled content of the PI pool while not affecting that of PC (data not shown), indicating that this lipid may serve as a major source for AA. To further delineate the type of PL\( A_2 \) that is involved in the \( H. pylori \)-induced AA release, the effect of the iPLA\(_2\)-specific inhibitor HELSS (33) and the secretory PL\( A_2 \) inhibitor aristolochic acid (34) was tested. \( H. pylori \)-induced AA release from the cells was not affected by aristolochic acid (50 \( \mu \)M) (data not shown). Likewise, HELSS at 10 \( \mu \)M did not affect the AA release, whereas higher concentrations (50 \( \mu \)M) inhibited both basal and \( H. pylori \)-induced AA release (data not shown), probably by inhibiting other important effectors in the signal transduction (35). These findings support a role of cPLA\(_2\) in the AA release.

\( H. pylori \) induces an increase of cytosolic Ca\(^{2+}\) when colonizing epithelial cells (19), and intracellular Ca\(^{2+}\) regulates cPLA\(_2\) activity; therefore, we studied the role of Ca\(^{2+}\) in AA release. Chelating intracellular calcium by preincubation of cells with BAPTA/AM (200 and 400 \( \mu \)M) abolished release of AA in response to \( H. pylori \) (Fig. 2C). Taken together, these results strongly suggest that AA release stimulated by \( H. pylori \) is Ca\(^{2+}\)-dependent.

The release of AA from membrane phospholipids could also occur through PLC activation followed by the action of the DAG lipase (23). However, \( H. pylori \)-induced AA release from the cells was insensitive to inhibition by U73122 (1 and 10 \( \mu \)M) (Fig. 2D), an inhibitor of the PLC (36). Unexpectedly, U73122 itself caused an increase in basal AA release, thereby increasing the AA formation in \( H. pylori \)-infected cells. Under the same experimental conditions, \( H. pylori \) caused a 1.2- and 2-fold increase of total inositol phosphates (mono-, bis-, and trisphosphate) in myo-[\(^{3}H\)]inositol-labeled HeLa and AGS cells, respectively, which was prevented by the PLC inhibitor U73122 (results not shown), demonstrating that the failure of the inhibitor to block \( H. pylori \)-induced AA release is not the result of U73122 failing to inhibit PLC. RHC80267 (40 and 80 \( \mu \)M), an inhibitor of the DAG lipase (37), slightly inhibited the basal release of AA from cells by about 30% but had no effect on \( H. pylori \)-induced AA release (Fig. 2E). Therefore, PLC and DAG lipase signaling pathways are not involved in the \( H. pylori \)-induced AA release.

Another potential pathway of AA release involves activation of PLD (24). To test this hypothesis, the release of water-
TABLE I

Effect of H. pylori on AA release and phospholipid hydrolysis in [14C]AA-labeled HeLa cells

| Treatment | Lipid |
|-----------|-------|
|           | PA    | PS/PI | PC    | PE    | AA | NL |
| 0-min control | 0.06 ± 0.01 | 10.22 ± 0.93 | 33.07 ± 1.26 | 36.90 ± 3.40 | 0.54 ± 0.13 | 9.80 ± 3.33 |
| 30-min control | 0.06 ± 0.01 | 6.86 ± 1.24 | 32.84 ± 1.25 | 37.97 ± 0.97 | 0.71 ± 0.13 | 9.87 ± 3.56 |
| 60-min control | 0.05 ± 0.01 | 9.66 ± 1.06 | 32.00 ± 1.14 | 37.48 ± 3.37 | 0.85 ± 0.21 | 10.18 ± 3.60 |
| 180-min control | 0.05 ± 0.01 | 9.77 ± 1.66 | 31.43 ± 0.51 | 37.79 ± 3.18 | 1.13 ± 0.22 | 7.87 ± 3.74 |
| 30-min (P12) | 0.12 ± 0.02* | 5.83 ± 0.99* | 31.77 ± 0.71 | 41.67 ± 3.72 | 1.37 ± 0.24* | 10.47 ± 3.50 |
| 60-min (P12) | 0.15 ± 0.03* | 5.56 ± 1.15* | 30.96 ± 1.26 | 41.16 ± 3.85* | 3.04 ± 0.33* | 10.11 ± 3.07 |
| 180-min (P12) | 0.19 ± 0.05* | 7.85 ± 1.63* | 27.36 ± 1.40* | 43.18 ± 4.20* | 3.69 ± 0.32* | 6.99 ± 2.55 |
| 30-min melittin | 0.28 ± 0.04* | 6.48 ± 1.56* | 30.97 ± 1.31* | 39.39 ± 4.88 | 8.78 ± 1.46* | 7.42 ± 2.48 |
| 60-min melittin | 0.21 ± 0.03* | 7.06 ± 1.43* | 30.08 ± 1.43* | 36.15 ± 4.48 | 9.80 ± 1.97* | 6.83 ± 2.88 |
| 180-min melittin | 0.14 ± 0.03* | 5.43 ± 2.09* | 28.53 ± 0.67 | 37.77 ± 3.37 | 9.00 ± 1.34* | 8.95 ± 2.29 |

* p < 0.05.

Effect of phospholipase inhibitors on H. pylori-induced AA release. HeLa cells were labeled as described in the legend to Fig. 1 and then reincubated in nonradioactive medium containing 1 mg/ml bovine serum albumin. Mepacrine (A), MAP (B), BAPTA/AM (C), U71322 (D), or RHC80267 (E) were added for 30 min. Cells were then exposed to H. pylori (P12) at a MOI of 200 or left untreated for another 60 min. The lipids were extracted from cells and their medium and analyzed by two-dimensional TLC. Results are expressed as a percentage of total lipid radioactivity and represent the means ± S.E. of five independent experiments (panels A, B, and E); results in panel C are the means ± S.E. from triplicate determinations in a representative experiment. The asterisks denote a significant difference compared with the respective response to H. pylori without drug pretreatment (p < 0.05).

The results are expressed as a percentage of total lipid radioactivity and represent the means ± S.E. of five independent experiments for H. pylori (P12) and three independent experiments for melittin. Significant differences compared with the untreated control were determined by Student’s paired t test. NL, neutral lipids.

CPLA2 Activation in Epithelial Cells by H. pylori

Results are expressed as a percentage of total lipid radioactivity and represent the means ± S.E. of five independent experiments for H. pylori (P12) and three independent experiments for melittin. Significant differences compared with the untreated control were determined by Student’s paired t test. NL, neutral lipids.

Effect of H. pylori on AA release and phospholipid hydrolysis in [14C]AA-labeled HeLa cells

Results are expressed as a percentage of total lipid radioactivity and represent the means ± S.E. of five independent experiments for H. pylori (P12) and three independent experiments for melittin. Significant differences compared with the untreated control were determined by Student’s paired t test. NL, neutral lipids.
modulating the H. pylori (P12) effects on AA release, HeLa cells were pretreated with 1 μg/ml PTX for 24 h. PTX alone did not have any effect on basal AA release, but PTX treatment significantly reduced the H. pylori-induced AA release (Fig. 4A). These results suggest that PTX-sensitive G-proteins are involved in mediating the stimulatory effect of H. pylori on AA release.

Next, we investigated whether protein kinase C and/or mitogen-activated protein kinases are involved in the H. pylori-induced AA release. Before colonization with H. pylori (P12), cells were pretreated with various inhibitors of protein kinase C; staurosporine (C), BIM (40), and K-252a (41). As shown in Fig. 4B, staurosporine (0.1 μM), BIM (2.5 μM), and K-252a (0.1 μM) failed to affect the AA release induced by H. pylori. Higher concentrations of BIM (10 μM) had no effect on H. pylori-induced AA release, whereas staurosporine (1 μM) and K252a (1 μM) increased the basal AA release and thereby the AA formation in H. pylori-infected cells (data not shown). Preincubation with a specific inhibitor of the extracellular signal-regulated kinase-activating pathway, PD98059 (42), had no significant effect on the AA release induced by H. pylori (P12) (Fig. 4C). We have shown previously that 50 μM PD98059 completely blocked the activation of the extracellular signal-regulated kinase pathway in response to H. pylori (17). In contrast, pretreatment of cells with SB202190 (10 and 20 μM), a specific inhibitor of the p38 stress-activated kinase (43), significantly decreased the release of AA induced by H. pylori (P12) (Fig. 4D). In agreement with the inhibition of the AA release by SB202190, H. pylori-induced activation (phosphorylation) of p38 kinase (Fig. 4E) and phosphorylation of cPLA2 (Fig. 4F) were blocked in the presence of the inhibitor. As a positive control, cells were treated with arsenite (0.5 mM) known to

![Table II](image)

**Table II**

Effect of H. pylori on the release of [14C]choline metabolites in HeLa cells

The amount of radioactivity released is expressed as a percentage of total radioactivity associated with cells and medium. The data are the means ± S.E. of three independent experiments for H. pylori (P12) and the means ± range of duplicate determinations in one experiment for 12-O-tetradecanoylphorbol-13-acetate (TPA).

| Treatment | Release of [14C]choline metabolites | % | |
|-----------|-------------------------------------|---|---|
| Control   | P12 (100 μM)                        |   |   |
| 30 min    | 2.10 ± 0.39                         | 2.06 ± 0.28 | 3.78 ± 0.11 |
| 60 min    | 3.44 ± 0.76                         | 3.38 ± 0.47 | 9.48 ± 0.21 |
| 180 min   | 11.01 ± 4.36                        | 10.97 ± 2.92 | 23.31 ± 0.24 |

![Fig. 4](image)

**Fig. 4. H. pylori-induced AA release is PTX-sensitive and involves the activity of p38 kinase.** HeLa cells were labeled for 24 h as described in the legend to Fig. 1 and pretreated with PTX (1 μg/ml, included during labeling) (A), with inhibitors of the protein kinase C (staurosporine, BIM, and K-252a (all for 30 min)) (B), with an inhibitor of the extracellular signal-regulated kinase-activating pathway (PD98059) (30 min) (C), or with a specific inhibitor of the p38 stress-activated protein kinase (SB202190) (30 min) (D). Therefore, H. pylori (P12) was added at a MOI of 200, and incubation was continued for another 60 min. The lipids were extracted from cells and their medium and analyzed by two-dimensional TLC. Data are expressed as a percentage of AA formation in control cells without drug pretreatment incubated under the same conditions. In panels A and C, data represent the means ± S.E. from three independent experiments. The asterisks denote a significant difference compared with the respective response to H. pylori without drug pretreatment (p < 0.05). In panel B, results shown are the mean of two experiments plus or minus the difference from the mean. E, HeLa cells were pretreated with SB202190 (20 μM, 30 min) and infected with H. pylori (P12) at a MOI of 200 for the indicated periods of time. Phosphorylation of p38 kinase was monitored from whole cell lysates using a p38 phospho-specific antibody (upper panel). As a loading control, the same blot was probed with an anti-p38 antibody (lower panel). F, the shift in electrophoretic mobility of cPLA2, indicating cPLA2 phosphorylation was monitored in an immunoblot using an anti-cPLA2 antibody. Lanes 1 and 4, cell lysates prepared from nonstimulated; lanes 2 and 5, arsenite (Ars)-stimulated (0.5 mM, 15 min); lanes 3 and 6, H. pylori (P12)-infected cells (MOI 200, 60 min).
increase AA release through phosphorylation of cPLA2 via p38 kinase (44).

**DISCUSSION**

Infection of epithelial cells by *H. pylori* induced a rapid generation of AA and the production of PGE2. The separation of cell-associated lipids by TLC demonstrated that the release of AA from epithelial cells, as substrate for the production of PGs. This enzyme has a high specificity for AA at the sn-2 position of phospholipids and requires for activation both elevation of the intracellular concentration of Ca2+ and a phosphorylation step (22, 46). We found, consistent with cPLA2 involvement, that pretreatment of cells with the cPLA2 inhibitor MAFP, which, although it also inhibits iPLA2, is selective for cPLA2 among known Ca2+-dependent phospholipases (33), blocked the release of AA. In contrast, the *H. pylori*-induced AA release was not affected by HELESS or aristolochic acid, indicating that iPLA2 and secretory PLA2 are not involved. The inhibitory effect of the intracellular calcium chelator BAPTA confirmed the involvement of a Ca2+-dependent PLA2 in the AA release. In addition, exposure of cells to *H. pylori* resulted in a decrease in the electrophoretic mobility of cPLA2, a finding consistent with cPLA2 phosphorylation (46), which is known to increase the catalytic activity of cPLA2 in vitro (22).

Several evidences were presented in this study ruling out the involvement of additional pathways in the generation of AA. First, although exposure of HeLa and AGS cells to *H. pylori* induced generation of myo-[^14]C-inositol phosphates, confirming previous findings (18), inhibition of inositol phosphate production by the PI-PLC inhibitor U73122 had no effect on AA release, indicating that PI-PLC is not involved. Second, no significant release of choline metabolites or a decrease of PC was detected when[^14]C-choline-labeled cells were exposed to *H. pylori*, ruling out the activation of PLD by *H. pylori*. This conclusion was supported by the observation that in the presence of ethanol, which substitutes for water in the transphosphatidylation reaction catalyzed by PLD, formation of PEt was not detectable in *H. pylori*-infected cells. Furthermore, formation of PEt would decrease the amount of PA, thereby inhibiting the AA release, if PA is the source for AA. However, inhibition of *H. pylori*-induced AA release was not observed for cells treated with ethanol. Moreover, wortmannin, known to inhibit PLD activation in a number of cell types (39), failed to affect the AA release induced by *H. pylori*. Finally, in the presence of the DAG lipase inhibitor RH50267, *H. pylori*-induced AA release was unimpeded.

The *H. pylori*-induced predominant hydrolysis of PI, which is exclusively located in the inner leaflet of the plasma membrane (47), suggests that the host epithelial cell membrane was not damaged by the *H. pylori* phospholipases A1, A2, or C (48–50) or sphingomyelinase (51). Moreover, by using the isogenic PAI mutant strain, which does not induce cPLA2 activation, we excluded the possibility that the bacterial phospholipases are able to activate host cell AA release from the epithelial cells, as has been shown in the case of the *Clostridium perfringens* α-toxin (52).

In a number of studies including ours, activation of cPLA2 has been shown to be PTX-sensitive, implying that members of the heterotrimeric Gα/Goα proteins are involved in its regulation (53–56). Although the underlying mechanism for the regulatory role of Gα/Goα proteins on cPLA2 activation is still unclear, it has been proposed that the βγ subunits released from Gα/Goα proteins can stimulate cPLA2 activity and AA release (57). Furthermore, it has been shown that mitogen-activated protein kinases are involved in cPLA2 activation (22, 46). Our studies, using specific inhibitors of protein kinase C and the extracellular signal-regulated kinase cascade, clearly demonstrated that the ability of *H. pylori* to cause a release of AA from epithelial cells does not depend on the activation of these kinases. This conclusion is supported by the observation that *H. pylori* induces the activation of the extracellular signal-regulated kinase cascade in a PAI-independent manner (14, 17), whereas in marked contrast to that, AA release induced by *H. pylori* is PAI-dependent. Recently, evidence for the involvement of p38 kinase in cPLA2 activation has been presented (44, 58–60). In *H. pylori*-stimulated cells, activation of p38 kinase, the mobility shift of cPLA2, and AA release were clearly abolished after pretreatment of cells with the p38 kinase inhibitor SB202190. These findings indicate the involvement of the p38 kinase in the signaling cascade leading to cPLA2 phosphorylation and AA release. Notably, SB202190 has been used to inhibit p38 kinase activated by various stimuli (60, 61) and thereby shown to reduce phosphorylation of cPLA2 in HeLa cells (59) as well as to block AA release in thrombin-stimulated platelets (61). Our findings are also consistent with the observation that activation of the p38 kinase by *H. pylori* in epithelial cells is strictly PAI-dependent (14). The release of AA was sensitive to both PTX and an inhibitor of p38 kinase, indicating that PTX-sensitive Gα/Goα proteins could be involved in p38 kinase activation. Hence, additional work is required to elucidate the signaling upstream of p38 kinase involved in the activation of cPLA2 by *H. pylori*.

In conclusion, in this study we have shown that colonization of epithelial cells by *H. pylori* promotes a rapid release of AA predominately from PI for PGE2 production via activation of cPLA2. The signaling pathway requires a pertussis toxin-sensitive G-protein and p38 stress-activated protein kinase but not the activation of PLC, PLD, protein kinase C, and extracellular signal-regulated kinase. The *H. pylori*-induced release of AA and PGE2 from epithelial cells and their role in the support or prevention of physiological and/or inflammatory reactions in the stomach remain to be elucidated. Release of AA for PG production by activation of cPLA2 could play an important role in mucosal defense to the bacterial infection (5, 62) since gastric epithelial cells are the first site of contact with *H. pylori*. On the other hand, prolonged activation of cPLA2 is likely to be damaging to the gastric epithelia by excessive degradation of membrane phospholipids releasing AA and lysophospholipids.

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**REFERENCES**

1. Bodger, K., and Crabtree, J.E. (1998) *Br. Med. Bull.* 54, 139–150
2. Blaser, M. J. (1987) *Gastroenterology* 93, 371–383
3. Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., Orentreich, N., Vogelman, J. H., and Friedman, G. D. (1994) *N. Engl. J. Med.* 330, 1267–1271
4. Wakabayashi, H., Orihara, T., Nakaya, A., Miyamoto, A., and Watanabe, A. (1998) *J. Gastroenterol. Hepatol.* 13, 566–571
5. Eberhart, C. E., and Dubois, R. N. (1995) *Gastroenterology* 108, 285–301
6. Kobayashi, K., and Arakawa, T. (1995) *J. Clin. Gastroenterol.* 21, Suppl. 1, 12–17
7. Covacci, A., Telford, J. L., Del Giudice, G., Parsonnet, J., and Rappuoli, R.
