Full Paper

Protein Kinase C Activation by *Helicobacter pylori* in Human Gastric Epithelial Cells Limits Interleukin-8 Production Through Suppression of Extracellular Signal-Regulated Kinase

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Abstract. *Helicobacter pylori* (*H. pylori*) infection of gastric epithelial cells has been shown to induce interleukin (IL)-8 production, but the signal transduction mechanism leading to IL-8 production has not been clearly defined. Here, we investigate the role of protein kinase C (PKC) in the mechanism of induction of IL-8 release by *H. pylori* in human gastric epithelial cells. In MKN45 cells, *H. pylori*-induced IL-8 release was enhanced by treatment with PKC inhibitors (GF109203X and calphostin C) and PKC depletion, which completely inhibited PKC activity. Moreover, PKC inhibitors and PKC depletion increased extracellular signal-regulated kinase (ERK) activity and phosphorylation, but not calcium/calmodulin-dependent protein kinase II (CaMK II) activity, in response to *H. pylori* infection. PKC activated by *H. pylori* inhibited activation of ERK induced by *H. pylori* without affecting the CaMK II activity and negatively regulated IL-8 production in human gastric epithelial cells.

Keywords: *H. pylori*, interleukin-8, protein kinase C, extracellular signal-regulated kinase, MKN45 cell

Introduction

Helicobacter pylori (*H. pylori*) plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and lymphoma arising from gastric mucosa-associated lymphoid tissue. Attachment of *H. pylori* to gastric epithelial cells leads to the production of chemokines, such as interleukin (IL)-8, which in turn cause activation and recruitment of neutrophils to the site of infection (1, 2). Several recent studies indicate that attachment of *H. pylori* to gastric epithelial cells induces IL-8 production through activation of multiple signaling pathways (3, 4). It is now known that nuclear factor (NF)- κ B activation plays a pivotal role in *H. pylori*-induced IL-8 production (5, 6). Moreover, we have recently reported that *H. pylori*-induced IL-8 production is dominantly regulated by Ca^{2+} /calmodulin signaling and that extracellular signal-regulated

kinase (ERK) plays an important role in the signal transmission for efficient activation of NF- κ B induced by *H. pylori* that results in IL-8 production (7).

Protein kinase C (PKC) is a family of protein-serine/threonine kinases that are believed to function as integrators of mitogenic signals originating from several classes of cell surface receptors. In gastric epithelial cells also, PKC has been reported to be involved in various signaling pathways (8, 9). As the precise intracellular signaling systems of PKC in *H. pylori*-stimulated IL-8 production have not yet been defined clearly, in the present study, we aimed to determine the interactive role in *H. pylori*-induced production of IL-8 through the ERK and NF- κ B pathway in human gastric epithelial cells. Here, we show that PKC activated by *H. pylori* negatively regulates IL-8 production induced by *H. pylori* at a point upstream from ERK and NF- κ B.

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Materials and Methods

Cell and *H. pylori* culture

The human gastric epithelial cancer cells, MKN45 (RIKEN Gene Bank, Tsukuba), were maintained in RPMI 1640 containing 10% fetal bovine serum (FBS), L-glutamine, 100 U/ml penicillin G, and 100 μ g/ml streptomycin. Cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂. For the experiments, cells at approximately 80% confluence in the culture wells were incubated in serum-free RPMI 1640 for 12 h to suppress serum-induced IL-8 release, unless otherwise stated. *H. pylori* (*cagA*⁺ strain 43504; American Type Culture Collection, Rockville, MD, USA) was plated on to blood agar supplemented with 5% FBS in a microaerobic atmosphere at 37°C. For the experiments, *H. pylori* was harvested from the plates and suspended in RPMI 1640 not containing FBS. The bacteria:cell ratio in the stimulation experiments ranged between 75:1 to 100:1.

Reagents

[γ -³³P]ATP (111 TBq/mmol) was purchased from New England Nuclear (Wilmington, DE, USA); rabbit polyclonal anti-*H. pylori* antibody and fluorescent isothiocyanate-conjugated anti-rabbit immunoglobulin G antibody from DAKO (Kyoto); polyclonal anti-inhibitor of κ B (I κ B) α antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and polyclonal anti-ERK antibody and polyclonal anti-phosphospecific-ERK antibody from New England Biolabs (Beverly, MA, USA). All the antibodies were dissolved in saline. GF109203X {2-[1-(3-dimethylaminopropyl)-1*H*-indol-3-yl]-3-(1*H*-indol-3-yl)-maleimide}, A-23187, KN-93 {2-[*N*-(2-hydroxyethyl)]-*N*-(4-methoxybenzenesulfonyl) amino-*N*-(4-chlorocinnamyl)-*N*-methylbenzylamine}, and PD98059 (2'-amino-3'-methoxyflavone) were purchased from Calbiochem (San Diego, CA, USA); and MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide), calphostin C, and 12-myristate 13-acetate (PMA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the inhibitors were dissolved in dimethyl sulfoxide and diluted with cell culture medium.

IL-8 measurement

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) at 37°C in serum-free RPMI 1640 for 6 h. The supernatants were then aspirated, centrifuged at 2,500 rpm for 10 min, and stored at -80°C until assay. IL-8 levels in the culture supernatants were determined with a specific enzyme-linked immunosorbent assay kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) using an

antibody against human IL-8, according to the manufacturer's instructions. The detection limit of this assay was 25.6 pg/ml.

ERK activity

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) for 60 min and A23187 (10 μ M) for 10 min at 37°C in serum-free RPMI 1640. The reaction was terminated by the replacement of medium with ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4, 20 mM NaCl, 2 mM EGTA, 2 mM dithiothreitol, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml leupeptin, and 10 μ g/ml aprotinin); the cell lysates were centrifuged at 14,000 $\times g$ for 10 min after brief sonication; and the supernatants were assayed with an ERK assay kit (Amersham Pharmacia Biotech) that measured the incorporation of [γ -³³P]ATP into synthetic peptide as a specific ERK substrate (7).

PKC activity

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) for 60 min and PMA (100 nM) for 10 min at 37°C in serum-free RPMI 1640. The reaction was terminated by the replacement of medium with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 2 mM EGTA, 2 mM EDTA, 0.25 M sucrose, 10 mM 2-mercaptoethanol, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml leupeptin, and 10 μ g/ml aprotinin), and the cell lysates were centrifuged at 100,000 $\times g$ for 60 min after brief sonication. The pellets suspended in assay buffer containing 1% noindet P-40 were stored on ice for 1 h, and the measurement was conducted with a PKC assay kit (Amersham Pharmacia Biotech) that measured the incorporation of [γ -³³P]ATP into synthetic peptide as a specific PKC substrate (10).

Calcium/calmodulin-dependent protein kinase II (CaMK II) activity

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) for 5 min and A23187 (10 μ M) for 2 min at 37°C in serum-free RPMI 1640. The reaction was terminated by the replacement of medium with ice-cold lysis buffer (20 mM PIPES, pH 7.0, 0.5 mM EGTA, 1 mM EDTA, 1 mM Na₃VO₄, 0.4 mM ammonium molybdate, 1 mM phenylmethylsulfonyl fluoride, 10 μ g/ml leupeptin, 10 μ g/ml aprotinin, 0.1% Triton X-100); the cell lysates were centrifuged at 14,000 $\times g$ for 5 min after brief sonication; and the supernatants were assayed with a CaMK II assay kit (Life Technologies, Gaithersburg, MD, USA) that measured the incorporation of [γ -³³P]ATP into synthetic peptide (autocamtide 3) as a specific CaMK II substrate (11).

MTT assay for cellular viability

After culture for 6 h in the presence of inhibitors, MTT at 5 mg/ml was added to each well for a contact period of 2 h. After the formation of formazan crystals, the culture medium supernatant was removed from the wells without disruption of the formazan precipitate. The absorbance was measured at 570 nm using a microplate spectrophotometer (SPECTRAMax 340; Molecular Devices, Sunnyvale, CA, USA). It was shown previously that the viable cell numbers correlated well with the optical density as determined by the MTT assay (7).

H. pylori adherence assay

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) at 37°C in serum-free RPMI 1640 for 6 h. The medium and unbound *H. pylori* were discarded, and the cells with *H. pylori* were washed with assay medium and fixed with 10% formaldehyde. They were incubated with a 1:10 dilution of rabbit anti-*H. pylori* antibody at 4°C and washed with PBS. They were further incubated with a 1:20 dilution of fluorescent isothiocyanate-conjugated pig anti-rabbit immunoglobulin G antibody. Fluorescence was determined using a fluorescent plate reader (Cytofluor II; PerSeptive Biosystems, Tokyo) (12).

Western blotting

Cells were stimulated with *H. pylori* (2×10^7 CFU/ml) at 37°C in serum-free RPMI 1640 for the specified durations. The reaction was terminated by the replacement of medium with 1 ml ice-cold buffer, pH 7.2, containing 20 mM HEPES, 1.5 mM MgCl₂, 420 mM NaCl, 0.2 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 1 mM Na₃VO₄, and 1 mM NaF. The cell lysates were then centrifuged at $14,000 \times g$ for 10 min, and the cytosolic fractions were applied for electrophoresis on 10% SDS gels. Proteins in the gel were transferred to a polyvinylidene difluoride membrane by electroblotting. The membrane was treated with anti-IκBα antibody, anti-ERK antibody, or anti-phospho-specific ERK antibody. After incubation with secondary anti-rabbit antibodies, immunoreactive proteins were detected by ECL (enhanced chemiluminescence) systems (Amersham Pharmacia Biotech) (7).

Statistical analysis

Statistical analysis was conducted by a one-way analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons using the SAS statistical program. Statistical significance was accepted at $P < 0.05$. The data were shown as the mean $\pm$ S.E.M.

Results

Enhancement of *H. pylori*-induced IL-8 production by PKC inhibition

To determine the effect of PKC on IL-8 production in response to *H. pylori* infection, MKN45 cells were preincubated with GF109203X and calphostin C, both PKC inhibitors, before stimulation by *H. pylori*. These inhibitors significantly enhanced *H. pylori*-induced IL-8

Fig. 1. Effects of PKC inhibitors on *H. pylori*-induced IL-8 production, IκBα degradation, and PKC activity. A: MKN45 cells were pretreated with GF109203X (GF) or calphostin C (CalC) for 30 min or with PMA (100 nM) for 24 h; and then they were cocultured with *H. pylori* (2×10^7 CFU/ml) for 6 h. B: Cells were cocultured with *H. pylori* (2×10^7 CFU/ml) for 1 h, and the cell lysates were analyzed by Western blotting using anti-IκBα antibody. C: Cells were pretreated with GF109203X (GF) or calphostin C (CalC) for 30 min and then cocultured with *H. pylori* (2×10^7 CFU/ml) for 1 h, or PMA (100 nM) for 10 min. Results in panels A and C are the mean $\pm$ S.E.M. of 4 experiments. Blots are representative of 3 separate experiments. Asterisks indicate a significant difference from the *H. pylori*-treated vehicle (* $P < 0.05$, ** $P < 0.01$) and PMA-treated vehicle (## $P < 0.01$).

production (Fig. 1A). The stimulatory effects of GF109203X and calphostin C were dose-dependent between 0.1 and 1 μ M and between 0.03 and 0.3 μ M, respectively. We also examined the effects of long-term (24 h) pretreatment with PMA before *H. pylori* infection on IL-8 production. Figure 1C showed that 24-h pretreatment with PMA downregulates PKC in MKN45 cells. The effect of *H. pylori* on IL-8 production was significantly enhanced in PKC-downregulated MKN45 cells (Fig. 1A) as compared with that in the cells not subjected to PMA pretreatment (Fig. 1A). Treatment with PMA for 10 min resulted in production of IL-8 in MKN45 cells, and GF109203X and calphostin C completely suppressed IL-8 production (Fig. 1A). *H. pylori* facilitated the degradation of $\text{I}\kappa\text{B}\alpha$, and GF109203X and calphostin C enhanced the *H. pylori*-induced $\text{I}\kappa\text{B}\alpha$ degradation in a dose-dependent manner (Fig. 1B). To confirm that GF109203X and calphostin C inhibited PKC in MKN45 cells, we observed the PKC activity after *H. pylori* stimulation. Pretreatment of the cells with the PKC inhibitors completely suppressed *H. pylori*- and PMA-mediated PKC activity (Fig. 1C).

Effect of PKC inhibitors on cell viability and H. pylori adhesion to cells

To confirm that the pharmacological inhibitors employed were not toxic to the MKN45 cells, the cell viability was assessed by the MTT assay, which measures mitochondrial function. GF109203X (1 μ M) and calphostin C (0.3 μ M) had no significant effect on the cell viability, as indicated by the equivalent mitochondrial activities in the control and inhibitor-treated cells under the conditions used in this study (Table 1). In addition, treatment with these inhibitors also had no significant effect on the adhesion of *H. pylori* to MKN45 cells (Table 1).

Enhancement of H. pylori-induced ERK activity by PKC inhibition

Pretreatment of MKN45 cells with GF109203X (0.1 – 1 μ M), calphostin C (30 – 300 nM), and PMA (0.1 μ M) long-term (24 h) before *H. pylori* infection enhanced both ERK activity and phosphorylation (Fig. 2: A and

Fig. 2. Effects of PKC inhibitors on *H. pylori*- or A23187-induced ERK activity and $\text{I}\kappa\text{B}\alpha$ degradation. A: MKN45 cells were pretreated with GF109203X (GF), calphostin C (CalC), or PD98059 (PD) for 30 min or with PMA (100 nM) for 24 h, and then they were cocultured with *H. pylori* (2×10^7 CFU/ml) for 1 h or A23187 (10 μ M) for 10 min. B: Cells were cocultured with *H. pylori* (2×10^7 CFU/ml) for 1 h or A23187 (10 μ M) for 10 min, and the cell lysates were analyzed by Western blotting using anti-phospho-ERK antibody. The cell lysates were also probed with anti-ERK antibody to confirm equal loading of cell protein. C: Cells were pretreated with GF109203X (GF), calphostin C (CalC) or PD98059 (PD) for 30 min or with PMA (100 nM) for 24 h, and then they were cocultured with *H. pylori* (2×10^7 CFU/ml) for 1 h or A23187 (10 μ M) for 10 min. The cell lysates were analyzed by Western blotting using anti- $\text{I}\kappa\text{B}\alpha$ antibody. Results in panel A are the mean $\pm$ S.E.M. of 5 experiments. Blots are representative of 3 separate experiments. Asterisks indicate a significant difference from the *H. pylori*-treated vehicle (* P <0.05, ** P <0.01) and A23187-treated vehicle (## P <0.01).

Table 1. Effects of PKC inhibitors on cell viability and *H. pylori* adhesion

	Vehicle	GF109203X	Calphostin C
Cell viability (optical density)	0.85 $\pm$ 0.18	0.81 $\pm$ 0.11	0.84 $\pm$ 0.10
Adhesion activity (fluorescent intensity)	78.4 $\pm$ 11.7	81.1 $\pm$ 8.6	83.8 $\pm$ 10.5

MKN45 cells were pretreated with GF109203X (1 μ M) and calphostin C (0.3 μ M), and then the MTT assay and *H. pylori* adherence assay were performed to assess cell viability and adhesion activity, respectively. Results are the mean $\pm$ S.E.M. of 3 experiments.

Fig. 3. Effects of PKC inhibitors on *H. pylori*- or A23187-induced CaMK II. MKN45 cells were pretreated with GF109203X (GF), calphostin C (CalC), or KN-93 (KN) for 30 min or with PMA (100 nM) for 24 h, and then they were cocultured with *H. pylori* (2×10^7 CFU/ml) for 5 min or A23187 (10 μ M) for 2 min. Results are the mean $\pm$ S.E.M. of 4 experiments. Asterisks indicate a significant difference from the *H. pylori*-treated vehicle (* $P < 0.05$) and A23187-treated vehicle (# $P < 0.05$).

B). The amount of $I\kappa B\alpha$ in MKN45 cells decreased after *H. pylori* stimulation. Both PKC inhibitors and PKC depletion induced more significant degradation of $I\kappa B\alpha$ as compared with *H. pylori* stimulation alone (Fig. 2C). The calcium ionophore A23187 (10 μ M) stimulated ERK activity and $I\kappa B\alpha$ degradation in MKN45 cells, both of which were completely inhibited by the MEK inhibitor PD098059 (30 μ M).

No enhancement of *H. pylori*-induced CaMK II activity by PKC inhibition

As shown in Fig. 3, GF109203X (1 μ M) and calphostin C (300 nM) had no effect on CaMK II activity induced by *H. pylori* infection. In addition, PKC depletion induced by long-term PMA (0.1 μ M) treatment also did not change the activity, although *H. pylori*- and A23187 (10 μ M)-induced CaMK II activity was completely inhibited by the CaMK II inhibitor KN-93 (10 μ M) (Fig. 3).

Discussion

Recently, we and others demonstrated the signaling pathway for *H. pylori* infection-induced IL-8 production through NF- κ B activation involving the ERK cascade in gastric epithelial cells (7, 13). However, the intracellular roles of PKC in *H. pylori*-stimulated IL-8 production had not been defined clearly. In the present study, we

observed that PKC activated by *H. pylori* inhibited *H. pylori*-induced activation of ERK without affecting the CaMK II activity and negatively regulated the IL-8 production in human gastric epithelial cells.

We reported previously that *H. pylori* significantly enhanced IL-8 production in a time-dependent manner for up to 24 h, and that the production of IL-8 was also dependent on the number of bacterial cells, within the range of 1×10^6 to 1×10^8 CFU/ml (7). Treatment with 2×10^7 CFU/ml *H. pylori* for 6 h resulted in sub-maximal production of IL-8 in MKN45 cells, whereas in the absence of such stimulation, no significant production of IL-8 was observed. Therefore, in this study, stimulation with 2×10^7 CFU/ml *H. pylori* for 6 h was employed for the IL-8 release experiments. In this study, GF109203X and calphostin C, both PKC inhibitors, completely blocked both *H. pylori*- and PMA-induced PKC activity; and these compounds enhanced *H. pylori*-induced IL-8 production in MKN45 cells without affecting the adhesion of *H. pylori* to the cells or the cell viability under our experimental conditions. Furthermore, the stimulatory effect of *H. pylori* on IL-8 production was markedly amplified in PKC down-regulated cells as compared with that in intact cells. These results suggest that *H. pylori*-induced IL-8 production is inhibited by *H. pylori*-induced PKC activation in MKN45 cells.

Accumulating evidence indicates the effects of PKC inhibitors or PKC depletion on the *H. pylori*-induced IL-8 production. Aihara et al. (3) and Yakabi et al. (14) reported that *H. pylori*-induced IL-8 release in gastric epithelial cells was not affected by treatment with staurosporine, a PKC inhibitor. On the other hand, Kassai et al. (15) showed that H-7 and calphostin C blocked *H. pylori*-induced IL-8 production at high concentrations (16, 17). PKC has more than ten isoforms, consisting of three subfamilies. Thus, it could be alternatively possible that the discrepancy in the observations is attributable to the different PKC isoforms expressed, depending on the cell type.

As shown in Fig. 1A and C, IL-8 release induced by *H. pylori* is negatively regulated by PKC activation in MKN45 cells since *H. pylori*-induced IL-8 release is increased by PKC inhibitors and PKC depletion. On the other hand, IL-8 release stimulated by PMA for 10 min is dependent on PKC activation since PMA-induced IL-8 release is completely blocked by PKC inhibitors. The discrepancy is probably due to the difference of intracellular signaling mechanisms regarding *H. pylori*- and PMA-induced IL-8 production or release because IL-8 release is stimulated by several factors such as calcium ionophore and some cytokines, as well as *H. pylori* and PMA (Fig. 4).

Fig. 4. Differential signaling process between *H. pylori* and PMA. –, inhibitory effect.

According to observations in previous studies (5, 6), we reported that NF- κ B activation plays a pivotal role in *H. pylori*-induced IL-8 production (7). NF- κ B is sequestered in the cytosol in a complex with the inhibitor protein I κ B α , and it is released and activated after phosphorylation of I κ B α , which regulates its ubiquitin-dependent degradation (18). In this study, we assessed the degradation of I κ B α protein to assess NF- κ B activation. *H. pylori* infection resulted in marked degradation of I κ B α , and GF109203X and calphostin C enhanced this degradation induced by *H. pylori* infection. Collectively, our findings suggest that PKC activated by *H. pylori* limits the overproduction of IL-8 in MKN45 cells.

We further investigated the transduction pathways after *H. pylori*-induced activation of PKC. In osteoblast-like cells, Miwa et al. (19) reported that PKC activated by IL-1 α negatively regulates IL-6 release induced by IL-1 α itself at a point upstream from ERK. Recently, we demonstrated that in gastric epithelial cells, ERK plays an important role in signal transmission for efficient *H. pylori*-induced NF- κ B activation that results in the production of IL-8 (7). To determine the signaling cascade leading to reduction of IL-8 production in response to PKC activation, we tested whether ERK signaling is involved in the pathway. In the present study, both PKC inhibitors and PKC depletion enhanced ERK activity and phosphorylation in response to *H. pylori* infection and stimulated degradation of I κ B α to a greater extent as compared with *H. pylori* stimulation alone. Thus, our data indicate that ERK activity is negatively regulated by PKC in the intracellular signaling triggered by *H. pylori* infection in MKN45 cells and that the attenuation of ERK by *H. pylori* infection results in suppression of the oversynthesis of IL-8 through the I κ B α /NF- κ B pathway.

Although NF- κ B activation is regulated by PKC in the inflammatory process (20), few reports indicated the negative effect of PKC on the I κ B/NF- κ B pathway. Our finding indicated that PKC is a negative regulator for NF- κ B through ERK inactivation and hence negatively regulates IL-8 release induced by *H. pylori*. This

discrepancy may be due to a specific effect of *H. pylori* or because the activation of specific isoforms of PKC is related to the negative regulation of ERK activity and subsequently NF- κ B signaling. It is well-known that conventional and novel, but not atypical, PKC subfamilies are activated by PMA, leading to cytokine production, so activation of an atypical PKC isoform by *H. pylori* might lead to the different effect to ERK and NF- κ B from other PKC subfamilies. Although the underlying mechanism for NF- κ B signal following PKC activation is unclear, both PKC and ERK are activated in *H. pylori*-infected MKN45 cells, and they may exert opposite effects on IL-8 production through the I κ B/NF- κ B pathway. However, further studies are needed to clarify the physiological role of PKC against IL-8 production through I κ B/NF- κ B pathway.

We, as also several others, have suggested that IL-8 production induced by *H. pylori* infection is mediated by Ca²⁺/calmodulin-dependent protein kinase (7, 21, 22). Thus, we examined whether CaMK II activation by *H. pylori* is involved in the signaling pathway of IL-8 production. In the present study, although KN-93, a CaMK II inhibitor, blocked CaMK II activity induced by A23187 or *H. pylori*, neither PKC inhibitors nor PKC depletion affected CaMK II activation in response to *H. pylori* infection. Our results indicate that at least CaMK II activity is independent of PKC-sensitive reduction of IL-8 release induced by *H. pylori* (Fig. 4). The mechanisms by which PKC might suppress ERK activation remain to be established. One possibility is that PKC regulates SH2-containing tyrosine phosphatase 2 (SHP-2), resulting in an abnormal cellular response. SHP-2 can be phosphorylated by PKC (23). Also, *H. pylori* infection of gastric epithelial cells induces SHP-2 phosphorylation and perturbs cellular functions (24). However, several studies have suggested a positive role of SHP-2 in the ERK pathway (25, 26). Thus, it is unlikely that SHP-2 is a direct candidate, and further studies are required to identify a PKC pathway that results in ERK activation.

In conclusion, we show that *H. pylori* infection activates PKC in human gastric epithelial cells and that the activated PKC then negatively regulates IL-8 release induced by *H. pylori* at a point upstream from ERK and downstream of CaMK II.

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