Abstract. *Helicobacter pylori*-initiated chronic gastritis is characterized by the cytotoxin-associated gene (Cag) pathogenicity island-dependent upregulation of pro-inflammatory cytokines in gastric epithelial cells, which is largely mediated by the activation of nuclear factor (NF)-κB as a transcription factor. However, the precise regulation of NF-κB activation, particularly post-translational modifications in the CagA-induced inflammatory response, has remained elusive. The present study showed that *Helicobacter pylori* CagA, an important virulence factor, induced the expression of P300/CBP-associated factor (PCAF) in gastric epithelial cells. Further study revealed that PCAF was able to physically associate with the NF-κB p65 sub-unit and enhance its acetylation. More importantly, PCAF-induced p65 acetylation was shown to contribute to p65 phosphorylation and further upregulation of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in gastric adenocarcinoma cells. In conclusion, the results of the present study indicated that *Helicobacter pylori* CagA enhanced TNF-α and IL-6 in gastric adenocarcinoma cells through PCAF-mediated NF-κB p65 sub-unit acetylation.

Introduction

Infection with *Helicobacter pylori* is one of the most common types of bacterial infection. *Helicobacter pylori* infection mainly occurs in economically underdeveloped regions, with the infection rates of pediatric patients in China, Japan and Korea being higher than those in developed countries (1-5). *Helicobacter pylori* colonizes the stomach and duodenum areas, causing chronic inflammation of the gastric mucosa, and the development of stomach ulcers and neoplasms (gastric cancer and mucosa-associated lymphoid tissue) (6,7). However, the mechanism by which *Helicobacter pylori* infection causes pathological changes of the gastric mucosa remains to be fully elucidated.

Inflammatory responses have been known to have a key role in the pathogenesis of *Helicobacter pylori* infection and contribute to chronic gastritis as well as gastric and duodenal ulcers in the patients (8-10). A growing body of evidence has demonstrated that elevated levels of inflammatory factors, including tumor necrosis factor (TNF)-α, interferon-γ, interleukin (IL)-6, IL-8 and IL-32 were associated with more serious pathogenesis of patients infected with *Helicobacter pylori* (10-15). However, the underlying regulatory mechanisms of the production of inflammatory factors have largely remained elusive.

Nuclear factor (NF)-κB has an important role in the regulation of inflammatory responses in mammals (16,17). It has been demonstrated that *Helicobacter pylori* infection activates NF-κB and its target genes, particularly inflammatory factors in epithelial cells, which is thought to be critical for *Helicobacter pylori*-initiated chronic inflammation (9,18-21). The virulence factor cytotoxin-associated gene A (CagA) encoded by the *Helicobacter pylori* Cag pathogenicity island, has an important role in the pathogenicity of *Helicobacter pylori*, including *Helicobacter pylori*-induced activation of NF-κB and expression of NF-κB target genes (22,23). However, the exact function of CagA in the activation of NF-κB and the NF-κB-dependent inflammatory response has not been well characterized.

Emerging evidence indicated that post-translational modification, such as acetylation, has important roles in various biological events, including inflammation (24-26). It has been reported that the acetylation of the NF-κB p65 sub-unit has an important role in its activation (27-29). In addition, recent studies showed that P300/CBP-associated factor (PCAF), also frequently referred to as lysine (K) acetyltransferase 2B, is a transcription co-activator that contains several nuclear
receptor-interacting domains and can function as an acetyl
transferase (30-33). PCAF has also been reported to be associ-
ated with infection and inflammation (34-37). Therefore, there
is a requirement to explore the roles of PCAF in mediating p65
activation and the production of TNF-α and IL-6 in gastric
adenocarcinoma cells.

The present study aimed to investigate the implication of Helicobacter pylori CagA in the inflammatory response of
human gastric adenocarcinoma AGS cells in vitro, as well as
PCAF-mediated p65 acetylation and its role in regulating the
production of TNF-α and IL-6 as two important inflammatory
factors in AGS cells.

Materials and methods

Reagents. Monoclonal rabbit antibodies against human total
p65 (cat. no. 8242), phospho (p)-p65 (cat. no. 3031), as well as
monoclonal mouse antibodies against acetylated-Lysine
(cat. no. 9681) and human β-actin (cat. no. 3700) were from
Cell Signaling Technology (Danvers, MA, USA). Mouse mono-
clonal antibodies against human PCAF (cat. no. sc-13124) and
Hemagglutinin (HA)-tag (cat. no. sc-7392) were purchased from
Santa Cruz Biotechnology (Dallas, TX, USA). Western blot
analysis, horseradish peroxidase-conjugated anti-mouse immu-
munoglobulin G (IgG) antibody (cat. no. 7076) and anti-rabbit IgG
antibody (cat. no. 7074) were purchased from Cell Signaling
Technology. Enhanced chemiluminescence (ECL) Western
Blotting Substrate, co-immunoprecipitation (co-IP) assay buffer,
radioimmunoprecipitation assay (RIPA) lysis buffer, a bicine
nolinic acid (BCA) protein assay kit and protein G-Sepharose
beads were purchased from Thermo Fisher Scientific (Waltham,
MA, USA). Polyvinylidene difluoride (PVDF) membranes were
obtained from Roche (Basel, Switzerland). TRIzol reagent was
purchased from Invitrogen Life Technologies (Carlsbad, CA,
USA). Moloney Murine Leukemia Virus reverse transcriptase
and oligo (dT) 15 primer were purchased from Promega
(Madison, WI, USA). TaqMan® Fast Advanced Master Mix
was from Applied Biosystems (Thermo Fisher Scientific).
The plasmids pGCSI-U6/neo/green fluorescence protein (GFP)
were obtained from Shanghai Genkan Biotechnology Co., Ltd.
(Shanghai, China). The pcDNA3.1 vector was purchased from
Shanghai Genkan Biotechnology Co., Ltd. (Shanghai, China).
The plasmids pGCSI-U6/neo/green fluorescence protein (GFP)
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were obtained from Shanghai Genkan Biotechnology Co., Ltd.
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Shanghai Genkan Biotechnology Co., Ltd. (Shanghai, China).

Cell culture and treatment. The human AGS cells were
obtained from the American Type Culture Collection
(Mannassas, VA, USA). Cells were cultured in the medium of
RPMI-1640 (Gibco-BRL; Invitrogen Life Technologies)
supplemented with 10% fetal bovine serum (FBS; Gibco-BRL)
and antibiotics (50 U/ml penicillin and 100 µg/ml strep-
tomycin) at 37°C in a 5%-CO2 incubator. AGS cells were
incubated with Helicobacter pylori CagA protein at 37°C
for different time points. For certain studies, the cells were
incubated with 10 µM PDTC at 30 min before treatment with
Helicobacter pylori CagA protein.

Construction of overexpression plasmids. The open
reading frame of human PCAF mRNA (National Center of
Biototechnology Information reference sequence,
NM_003884.4) containing a HA tag was amplified by poly-
merase chain reaction (PCR) from cDNA of human AGS
cells. The PCR products and pcDNA3.1 vector were further
digested with the two restriction enzymes of HindIII and XhoI,
and then ligated with each other by using T4 DNA ligase. The
recombinant plasmids were amplified in the Escherichia coli
strain DH5α at 37°C for 16 h. Subsequently, the plasmids
were extracted by QIAprep spin miniprep kit according to
the manufacturer's instructions. Finally, the constructed plas-
mids (pcDNA3.1/PCAF-HA) were sequenced to confirm the
nucleotide sequence by GenScript (Nanjing, China).

Construction of small hairpin (sh)RNA expression plasmids.
Various shRNA sequences targeting human PCAF mRNA
(NM_003884.4) were designed to silence the PCAF gene in
human AGS cells. The various DNA segments for the expres-
sion of PCAF shRNA were synthesized and inserted into the
shRNA pGCSI-U6/neo/GFP plasmids by Shanghai Genkan
Biotechnology Co., Ltd. The most effective shRNA expres-
sion plasmid to silence human PCAF gene was selected to be
used in subsequent experiments. The PCAF shRNA sequence
was as follows: AAGATGGCCGTGTATTGGGT and the
Scrambled shRNA sequence was as follows: AATGACGGG
CTTGTATTGGGT.

Cellular transfection. Plasmids were transfected into AGS
cells by using Lipofectamine 2000 according to the manu-
facturer's instructions. For transfection, 4 µg plasmid was
mixed with 250 µl serum-free RPMI-1640. At the same time,
10 µl Lipofectamine 2000 was mixed with 250 µl serum-free
RPMI-1640. The plasmids and Lipofectamine 2000 were
further incubated with each other for 20 min at room tempera-
ture. Finally, the 500-µl mixture was added into each well in
a 6-well plate containing 4x10³ AGS cells. The medium was
replaced with serum containing RPMI-1640 at 5 h after trans-
fection, and the cells were incubated sequentially.

Production of the CagA protein. Helicobacter pylori
CagA-His-tag was constructed in the pET21a plasmid from
Novagen (Madison, WI, USA). The plasmid was then trans-
formed into BL21(DE3) Singles™ Competent Cells (Novagen)
for protein expression and purification according to the manu-
facturer's instructions. The protein was extracted by B-PER®
bacterial protein extraction reagent (Thermo Fisher Scientific)
from 50 ml bacteria according to the manufacturer's instruc-
tions. Subsequently, HisPur™ Cobalt Spin Columns (Thermo
Fisher Scientific) were used for His-tag purification of the
Helicobacter pylori CagA protein according to the manufac-
turer's instructions.

co-IP experiment. AGS cells were lysed in co-IP assay buffer
at 4°C for 30 min. Cell lysates were then centrifuged at 15,000 x g
for 20 min at 4°C to remove any insoluble material. 300 µg cell
lysat was incubated with 40 µl protein G-Sepharose beads
in co-IP assay buffer at 4°C for 2.5 h under constant agita-
tion and then centrifuged at 1,000 x g for 2 min at 4°C. The
recovered supernatant was then incubated with the antibody
against human total p65 (1.5 µg for each sample) at 4°C overnight under constant agitation. The sample was then incubated with 40 µl protein G-Sepharose beads at 4°C for 2.5 h under constant agitation. Protein G-protein complex was precipitated by centrifugation and finally re-suspended in 40 µl SDS lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). The samples were then analyzed by SDS-PAGE followed by staining with specific antibodies against total p65 (1:1,000), acetylated-Lysine (1:1,000), PCAF (1:200) and HA (1:500), respectively. At the same time, 40-µg aliquots of whole-cell extract from the AGS cells were used to detect the protein expression as an input control.

RNA isolation and reverse transcription quantitative (RT-q)PCR. Cells were collected at the indicated time-points and RNA was extracted using TRIzol reagent. A total of 1 µg RNA was used for the first-strand cDNA synthesis using MMLV and oligo (dT) 15 primer according to the manufacturer’s instructions. The cDNA was amplified by using TaqMan® Fast Advanced Master Mix to detect the expression of human PCAF gene using primers purchased from GenScript (forward 5’-TACCTCGGTACGAA CCACA-3’ and reverse 5’-TCCTGTTCTGCTGTTCCAG-3’; Fam/Tamra-labeled probe, 5’-CGAGCGAAGCAATGTTCT CCCA-3’). The human β-actin gene was used as an internal control using primers purchased from GenScript (forward 5’-TGACCTCGAGCAAGAGATG-3’ and reverse 5’-GAA GGAAGGCTGGAAGAGTG-3’; Fam/Tamra-labeled probe, 5’-CGGCTGCTTCCAGCTCCTCC-3’). The 7500 Real-time PCR system (Applied Biosystems) was used to perform qPCR. The reaction program included an initial step for denaturation at 95°C for 10 min, and 40 cycles of denaturation at 95°C for 15 sec and annealing at 60°C for 60 sec. Each sample was assayed in triplicate. The relative levels of the gene expression were obtained using the 2^ΔΔCt method as described previously (38).
Western blot analysis. Cells were lysed in RIPA lysis buffer at 4°C for 30 min. The cellular lysates were then centrifuged at 15,000 x g for 20 min at 4°C to remove any insoluble material. The concentration of the protein was determined by a BCA protein assay kit according to the manufacturer’s instructions. The protein (40 µg/well) was subjected to 10-12% SDS-PAGE and transferred onto PVDF membranes using the Mini-Protean System (Bio-Rad Laboratories, Inc., Hercules, CA USA). The PVDF membranes were incubated in blocking buffer [5% skimmed milk in Tris-buffered saline containing Tween 20 (TBS-T)] at room temperature for 1 h and then incubated with the specific antibodies (anti-total p65, 1:1,000; anti-p-p65, 1:1,000; anti-acetylated-Lysine, 1:1,000; anti-PCAF, 1:200; anti-HA; 1:500) at 4°C overnight. β-actin expression in each sample was used as an internal standard (anti-β-actin, 1:1,000). After five washes with TBS-T, the PVDF membranes were further incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:2,000) or anti-rabbit IgG (1:2,000) at 37°C for 1 h. The bands were visualized using X-ray film (Life Technologies, Carlsbad, CA, USA) and the ECL detection system after washing the PVDF membranes five times. Finally, the density of the radiographic bands on the PVDF membranes was analyzed using the Quantity One software v4.4 (Bio-Rad Laboratories, Inc.).

ELISA. The levels of TNF-α and IL-6 in the cell media were determined using commercial human TNF-α and IL-6 ELISA kits (cat. no. 555212 and 550799) from BD Biosciences (Franklin Lakes, NJ, USA). ELISA was performed according to the manufacturer's instructions.

Figure 2. Activation of NF-κB promotes the production of TNF-α and IL-6 in human AGS cells exposed to CagA. (A) Western blot analysis showed that CagA (10 µg/ml) was able to induce the phosphorylation of NF-κB p65 subunit in human AGS cells at 1.5 h after stimulation. (B and C) Reverse transcription quantitative polymerase chain reaction and (D and E) ELISA showed that inhibition of NF-κB with PDTC significantly reduced the production of TNF-α and IL-6 in AGS cells induced by CagA (10 µg/ml for 4 h). Results shown are from one experiment, representative of three independent experiments. Values are expressed as the mean ± standard deviation (n=3 for each group). **P<0.01 compared to PBS group. NF, nuclear factor; TNF, tumor necrosis factor; IL, interleukin; PBS, dimethyl sulfoxide; DMSO, dimethyl sulfoxide; PDTC, pyrrolidine dithiocarbamate; p, phosphorylated; CagA, cytotoxin-associated gene A.

Figure 3. Acetylation of p65 is increased in human AGS cells stimulated by CagA. Human AGS cells were treated with 10 µg/ml CagA for 1.5 h and cell lysates were subjected to immunoprecipitation using anti-p65 antibodies. The levels of Ac-p65 and total p65 in immunoprecipitated complexes were detected by western blot analysis with specific anti-Ac-p65 and anti-p65 antibodies. β-actin without immunoprecipitation was used as a loading control. The results showed that the level of p65 acetylation was significantly enhanced in human AGS cells exposed to CagA. Results shown are from one experiment, representative of three independent experiments. Values are expressed as the mean ± standard deviation (n=3 for each group). **P<0.01 compared to PBS group. NF, nuclear factor; TNF, tumor necrosis factor; IL, interleukin; DMSO, dimethyl sulfoxide; PDTC, pyrrolidine dithiocarbamate; p, phosphorylated; CagA, cytotoxin-associated gene A.
Figure 4. Expression of PCAF and its association with p65 are enhanced in human AGS cells induced by CagA. (A) The expression of PCAF at the mRNA level was detected in human AGS cells induced with CagA (10 µg/ml for 1.5 h). Reverse transcription quantitative polymerase chain reaction analysis showed that CagA stimulation markedly increased the expression of PCAF mRNA in human AGS cells. (B) Anti-p65 antibodies were used for immunoprecipitation from lysates of human AGS cells stimulated with CagA (10 µg/ml for 1.5 h). The levels of PCAF and p65 in the immunoprecipitated complex were detected by western blot analysis with anti-PCAF and anti-p65 antibodies. Western blot analysis was also used to detect PCAF and β-actin expression in cell lysates without immunoprecipitation. The results showed that the molecular interaction of PCAF with p65 at the protein level was markedly enhanced in human AGS cells upon CagA stimulation. Results shown are from one experiment, representative of three independent experiments. Values are expressed as the mean ± standard deviation (n=3 for each group). **P<0.01 compared to PBS group. PCAF, P300/CBP-associated factor; CagA, cytotoxin-associated gene A; PBS, phosphate-buffered saline; IP, immunoprecipitation; IgG, immunoglobulin G.

Figure 5. PCAF induction promotes p65 acetylation in AGS cells stimulated by CagA. (A) AGS cells were transfected with PCAF shRNA for 36 h and then stimulated with 10 µg/ml CagA for 1.5 h. Cell lysates were then subjected to immunoprecipitation with anti-p65 antibodies. Western blot analysis was then used to detect the levels of Ac-p65 and total p65 in the immunoprecipitated complex with anti-Ac-p65c and anti-p65 antibodies. In addition, western blot analysis was also used to detect PCAF and β-actin expression in cell lysates without immunoprecipitation. The results showed that PCAF shRNA decreased CagA-induced p65 acetylation and phosphorylation in AGS cells. **P<0.01 compared to control shRNA + CagA group. (B) AGS cells were treated with pcDNA3.1 PCAF-HA overexpression vector for 48 h. Cell lysates were then subjected to immunoprecipitation with anti-p65 antibodies. Subsequently, the levels of PCAF-HA, Ac-p65 and total p65 in the immunoprecipitated complexes were detected by western blot analysis with anti-HA, anti-Ac-p65 and anti-p65 antibodies. The results showed that overexpression of PCAF triggered p65 acetylation and phosphorylation in AGS cells. **P<0.01 compared to pcDNA3.1 vector group. Results shown are from one experiment, representative of three independent experiments. Values are expressed as the mean ± standard deviation (n=3 for each group). CagA, cytotoxin-associated gene A; PCAF, P300/CBP-associated factor; PBS, phosphate-buffered saline; IP, immunoprecipitation; IgG, immunoglobulin G; shRNA, small hairpin RNA; Ac, acetylated; p, phosphorylated; HA, hemagglutinin tag.
Statistical analysis. All statistical analyses were performed using SPSS 12 software (SPSS, Inc., Chicago, IL, USA). Values are expressed as the mean ± standard deviation. The statistical significance of differences between groups was evaluated by one‑way analysis of variance with simultaneous multiple comparisons between groups by the Bonferroni method. P<0.05 was considered to indicate statistically significant differences.

Results

CagA promotes the activation of NF‑κB and the production of TNF‑α and IL‑6 in human AGS cells. Human AGS cells were cultured with purified Helicobacter pylori CagA and the production of TNF‑α and IL‑6 was subsequently observed. The results showed that CagA enhanced the production of TNF‑α and IL‑6 in human AGS cells in vitro in a dose- and time-dependent manner (Fig. 1). Furthermore, CagA was able to induce the activation of NF‑κB in human AGS cells in vitro (Fig. 2A). Of note, inhibition of NF‑κB significantly reduced the production of TNF‑α and IL‑6 in AGS cells induced by CagA (Fig. 2B‑E). These findings indicated that CagA promoted the production of TNF‑α and IL‑6 by human AGS cells in vitro via activation of NF‑κB.

CagA enhances p65 acetylation in human AGS cells. Since Helicobacter pylori CagA was found to have the ability to stimulate the activation of NF‑κB and the production of TNF‑α and IL‑6 in human AGS cells (Figs. 1 and 2), the effects of CagA stimulation on the acetylation of p65 were further assessed in AGS cells. The level of p65 acetylation was found to be significantly enhanced in human AGS cells exposed to
CagA in vitro (Fig. 3), suggesting that p65 acetylation may have an important role in promoting p65 activation in human AGS cells induced by CagA.

**PCAF and p65 association is increased in human AGS cells exposed to CagA.** The expression of PCAF at the mRNA and protein level was detected in human AGS cells induced by CagA. It was found that incubation with CagA significantly increased the mRNA and protein expression levels of PCAF in human AGS cells (Fig. 4A and B). Furthermore, the interaction of PCAF with p65 was assessed at the protein level by IP. The results showed that the molecular interaction of PCAF with p65 at the protein level was markedly enhanced in human AGS cells upon stimulation with CagA (Fig. 4B). These findings indicated that stimulation with CagA induced the expression of PCAF and further enhanced the molecular interaction of PCAF with p65 at the protein level in human AGS cells, suggesting a potential ability of PCAF to acetylate p65.

**PCAF induction contributes to p65 acetylation in AGS cells stimulated by CagA.** To further determine the role of PCAF expression in p65 acetylation and phosphorylation, AGS cells were treated with PCAF shRNA expression vector for 36 h followed by CagA stimulation for 1.5 h. A Co-IP assay showed that PCAF knockdown decreased CagA-induced p65 acetylation and phosphorylation in AGS cells (Fig. 5A). Conversely, overexpression of PCAF triggered p65 acetylation and phosphorylation in AGS cells (Fig. 5B). These results suggested that PCAF is necessary for p65 acetylation and phosphorylation in CagA-induced AGS cells.

**PCAF-mediated p65 acetylation is required for CagA-induced production of TNF-α and IL-6 in AGS cells.** Since PCAF was shown to be required for p65 acetylation in AGS cells stimulated by CagA (Fig. 5), the present study further explored the role of PCAF-mediated p65 acetylation in the production of TNF-α and IL-6 in AGS cells induced by CagA. The results showed that suppression of PCAF markedly reduced the production of TNF-α and IL-6 in AGS cells stimulated by CagA (Fig. 6A-D). Conversely, AGS cells overexpressing PCAF exhibited enhanced production of TNF-α and IL-6 (Fig. 6E-H). These results indicated that PCAF-mediated p65 acetylation is involved in the production of TNF-α and IL-6 in AGS cells stimulated with CagA.

**Discussion**

*Helicobacter pylori* is a Gram-negative microaerophilic bacterium that selectively colonizes in human gastric and duodenal mucosa. It is generally known that persistent *Helicobacter pylori* infection leads to chronic gastritis and severe gastric pathologies, including peptic ulcers and even gastric cancer (39-41). It has been reported that infection induces not only innate immune responses but also adaptive immune responses against microorganisms; however in most cases, this fails to eradicate the microorganisms. By contrast, permanent infection ultimately triggers chronic inflammation and further leads to the damage of the body (42,43).

It is well accepted that *Helicobacter pylori* infection triggers production of various cytokines, including IL-1β, IL-6, IL-8, IL-18, IL-32, TNF-α and IL-6, which are known to have important roles in chronic inflammation and ultimate disease outcome (10,14,44-46). Among these inflammatory cytokines, TNF-α and IL-6 are considered to be two important inflammatory factors that can also be produced in other chronic inflammatory diseases, including Crohn's disease, rheumatoid arthritis and atherosclerosis (47-49). These studies indicated that overproduction of TNF-α and IL-6 may have a key role in mediating the inflammatory response in the process of *Helicobacter pylori* infection. In addition, CagA is considered to be an important virulence factor for *Helicobacter pylori* infection. Therefore, the present study aimed to investigate the implication of *Helicobacter pylori* CagA in the inflammatory response of human AGS cells in vitro. The results revealed that CagA markedly stimulated the production of TNF-α and IL-6 in human AGS cells in vitro, which is in accordance with the findings of previous studies (50,51).

NF-κB is a major transcriptional factor that participates in the regulation of numerous cellular functions including inflammation (52-54). It has been demonstrated that *Helicobacter pylori* infection activates NF-κB and its target genes in gastric epithelial cells, which is thought to be critical for *Helicobacter pylori*-initiated chronic inflammation (9,21). The virulence factor CagA, encoded by the *Helicobacter pylori* Cag pathogenicity island, has an important role in the pathogenicity of *Helicobacter pylori*, including *Helicobacter pylori*-induced activation of NF-κB and expression of NF-κB target genes (22,23). However, the exact function of CagA in the activation of NF-κB and the NF-κB-dependent inflammatory response have not been well characterized. Emerging evidence indicated that post-translational modification, including ubiquitination and acetylation, has important roles in various biological events, including inflammation (55-60). Among these, the acetylation of the NF-κB p65 sub-unit has been shown to have an important role in its activation (27-29). The present study showed that the acetylation level of the NF-κB p65 sub-unit was significantly enhanced in human AGS cells incubated with CagA in vitro. Therefore, the present study aimed to investigate the regulatory mechanism of p65 acetylation in CagA-induced gastric adenocarcinoma cells.

Recent studies showed that PCAF functions as an acetyl transferase (30-33); furthermore, PCAF has been reported to be linked to infection and inflammation (34,35,61). However, the roles of PCAF in mediating p65 activation and the production of TNF-α and IL-6 in CagA-induced AGS cells has remained largely elusive. The present study therefore investigated the expression of PCAF in human AGS cells induced by CagA, and showed that CagA increased the mRNA and protein expression levels of PCAF in human AGS cells. Furthermore, the results of the present study demonstrated that PCAF was required for p65 acetylation and phosphorylation in human AGS cells stimulated with CagA, suggesting that p65 acetylation contributed to p65 phosphorylation. Of note, PCAF was demonstrated to have an important role in p65 acetylation, although PCAF expression was not strongly increased. This result indicated that, in addition to the expression of PCAF, the activation of PCAF may have had a predominant role in the regulation of p65 acetylation in human AGS cells stimulated with CagA.
In the present study, it was also demonstrated that PCAF-mediated p65 acetylation functionally contributed to the production of TNF-α and IL-6 in human AGS cells stimulated by CagA, indicating that PCAF may have an important role in promoting the production of inflammatory factors. Kiernan et al. [62] revealed that acetylation of p65 may have a key role in inhibitor of NF-κB-mediated attenuation of NF-κB transcription. The present study showed that p65 acetylation promoted its activation as a transcription factor to activate TNF-α and IL-6 gene expression. This difference may be due to different acetylated sites of p65. Therefore, further studies are required to identify the differential sites of PCAF-induced acetylation in AGS cells stimulated by CagA.

In conclusion, the results of the present study suggested that Helicobacter pylori CagA promoted the production of TNF-α and IL-6 in AGS cells through PCAF-mediated p65 acetylation. The present study therefore provided novel insight into the pathomechanism of Helicobacter pylori infection.

References


