

## *Helicobacter pylori* Interleukin- 8

### **Regulation of *Helicobacter pylori*-induced Interleukin-8 Production by Gastric Epithelial Cells *in vitro***

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**Background/Aims:** Production of interleukin-8 (IL-8) by gastric epithelial cells may play an important role in *H. pylori*-induced mucosal injury. The aim of this study was to evaluate physico-chemical factors and signal pathway regulating *H. pylori*-induced IL-8 production. **Methods:** Various physical and chemical pretreatments of *H. pylori* were performed before the stimulation of gastric epithelial cells such as AGS and KATO III. After pretreatment with protein kinase inhibitors, the production of IL-8 mRNA and protein were analyzed by reverse transcription-polymerase chain reaction and enzyme linked immunosorbent assay, respectively. To evaluate the possible synergism of tumor necrosis factor (TNF) secreted by epithelial cells, the pretreatment with anti-TNF blocking antibody was performed. **Results:** Significant decrease in IL-8 production was noted when *H. pylori* was pretreated with various physical and chemical methods. H89 (10  $\mu$ M) and calyculin A (25 nM) had no effect on IL-8 production, while staurosporin (1  $\mu$ M), herbimycin A (40 nM) or genistein (250  $\mu$ M) significantly inhibited the IL-8 production by epithelial cells. The IL-8 production induced by TNF and *H. pylori* was also inhibited by protein tyrosine kinase (PTK) inhibitors and protein kinase C (PKC) inhibitors. Anti-TNF antibody showed no significant effect on the *H. pylori*-induced IL-8 production. **Conclusions:** A live bacterium adhering to epithelial cell induces IL-8 production by epithelial cells. Two different protein kinases (PTK and PKC) may be involved in *H. pylori*-induced IL-8 production of epithelial cells. (**Kor J Gastroenterol 1999;33:601 - 614**)

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**Key Words:** *Helicobacter pylori*, Interleukin-8, Signal, Protein kinase

가

*Helicobacter pylori* (*H. pylori*) B 가

.1 *H. pylori* .1617 *H. pylori*  
protease, elastase

. *H. pylori* 18 IL-8

23 *H. pylori*가 *H. pylori* IL-8

가

45 가 가

*H. pylori* *H. pylori* IL-8 *H. pylori* 가

IL-8

.6 *H. pylori* 가  
tumor necrosis factor alpha (TNF ), interleukin-6 (IL-6), interleukin-8 (IL-8) 가  
kinase protein tyrosine kinase (PTK), protein kinase C (PKC), protein kinase A (PKA) protein phosphatase (inhibitor)

.7 *H. pylori* *H. pylori* IL-8 IL-8

.8-12 *H. pylori* IL-8

가

가 .9,11

*H. pylori* (sonificate) IL-8 1. *H. pylori*

13 1)

*H. pylori* KATO III (European Collection of Animal Cell Cultures, Salisbury, Wilts, U.K.) AGS (ATCC CRL 1739; American Type Culture Collection, Rockville, MD, U.S.A.) 100 g/ml penicillin-streptomycin 10% (fetal bovine serum; FBS, GibcoBRL, Grand Island, NY, U.S.A) RPMI 1640 8 5% CO2 37

adhesion pedestal4 *H. pylori* IL-8 IL-8 mRNA

*H. pylori* *H. pylori* lipo-polysaccharide (LPS), (water extractable surface protein), (urease) IL-1, TNF IL-8

.15 IL-8

24 well plate (Falcon, Becton Dickinson, Franklin lake, NJ, U.S.A.)  
. *H. pylori*

(penicillin-streptomycin)가  
2

2) *H. pylori*  
Cytotoxin/cagA ATCC 43504  
(NCTC 11637) . *H. pylori*

brain heart infusion agar (Oxoid)  
7% sheep blood, 1% IsoVitaleX (BBL, Becton Dickinson), 6 mg/L vancomycin, 20 mg/L nalidixic acid 2 mg/L amphotericin-B 가 7% sheep blood, 10 mg/L vancomycin, 5 mg/L trimethoprim lactate, 5 mg/L cefosulodin 5 mg/L amphotericin B 가 .  
37 3-5

가 10 *H. pylori*  
10 mM phosphate buffered saline (PBS; pH 7.4) MacFarland number 3 (9 × 10<sup>8</sup>/ml) 10% FBS  
가 70  
2

2. *H. pylori* TNF

24 well plate well 0.5 ml (1 × 10<sup>6</sup>/ml)  
well *H. pylori* 0.5 ml 가 37 , 5% CO<sub>2</sub> .  
0 , 1 , 5 , 15 , 30 , 60 , 120 , 240 , 300 가  
*H. pylori* (*H. pylori*: cell= 500:1). *H. pylori*  
IL-8 *H. pylori*  
가 2 1:1 1000:1  
TNF TNF (Recombinant human TNF , R&D system, Minneapolis, MN, U.S.A.) 0.01 unit/ml 100 unit/ml

가 2  
IL-8 stimulant phorbol myristate acetate (PMA)(Sigma, St. Louis, MO, U.S.A.)  
IL-8 mRNA IL-8

3. (reverse transcription-polymerase chain reaction; RT-PCR)

1) RNA  
*H. pylori* TNF  
RNA Chomczynski Sacchi<sup>19</sup>  
RNA Premate™(Bioneer, Seoul)  
2 g RNA 0.1 mg oligo (dT)15 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 mM dATP, dCTP, dGTP dTTP, 15 unit RNase inhibitor, 20 unit RNase free moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.) 20 ml  
57 10 , 42 1 , 94 5  
DNA (complimentary DNA; cDNA)

2) (polymerase chain reaction; PCR)  
cDNA 가 20 pM primer, 300 mM dNTP 0.5 U Taq polymerase (Perkin-Elmer Cetus, Emerville, CA, U.S.A.)가  
(Premix-RT/PCR™kit, Bioneer, Seoul)  
(10 mM Tris-HCl; pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub> 0.001% gelatin) 가  
DNA thermal cycler (Perkin Elmer 9600)  
95 1 denaturation  
, 72 2 annealing extension  
30  
cDNA RNA  
IL-8 primer 5'ATGACTTCCAAGCTGGCCGTGGC3', 5'CTCAGCCCTCTCAA-AAAC-TTCTC 3' .8 -actin

PCR . RT-PCR  
 1.5% agarose gel (FMC Bioproducts,  
 Rockland, ME, U.S.A.) ethidium  
 bromide densitometry  
 -actin (band) IL-8

4. IL-8

IL-8 (ELISA)  
 96 well microtiter plate  
 (Costar, Cambridge, MA, U.S.A.) borate buffered  
 saline (1.03% H3PO4, 0.73% NaCl; BBS) 200  
 goat anti-human IL-8 polyclonal immunoglo-  
 bulin G (pAb) (R&D System, Minneapolis, MN,  
 U.S.A.) well 100 µl  
 10 mM PBS (pH 7.4)/0.05% (v/v) Tween 20 3  
 . 0.5% BSA/PBS

(rhIL-8, R&D system) 가 2  
 . PBS/Tween 3 0.5%  
 BSA/PBS 400 rabbit anti-human IL-8  
 pAb (Endogen, Cambridge, MA, U.S.A.) well  
 100 µl 가 2 . PBS/  
 Tween 3 alkaline phosphatase 가  
 conjugate goat anti-rabbit IgG Ab (Jack-  
 son Laboratories, Avondale, PA, U.S.A.) 1000  
 100 µl 가 30 . PBS/  
 Tween 3 , Tris/NaCl 3  
 1 mg/ml disodium p-nitrophenyl phosphate (Life  
 Technologies, Gaithersburg, MD, U.S.A.) 가  
 15 (3% 2-propanol, 1 mM  
 iodinitrotetrazolium violet, 75 mg/ml, alcohol dehy-  
 drogenase 50 g/ml diaphorase; Life Technologies)  
 가 10 ELISA 492 nm  
 O.D.

5. *H. pylori*

IL-8

*H. pylori*  
 (5 × 10<sup>8</sup>/ml)

PBS

. , 0.5% formaldehyde 가  
 , 100 10 가  
 4 . *H.*  
*pylori* PBS .  
 (5 × 10<sup>5</sup>/ml)가  
*H. pylori* 가 3  
 IL-8 ELISA

*H. pylori*

IL-8

cell culture insert (0.4 µm pore size, P.E.T.  
 track-etched membrane; Falcon, MN, U.S.A.)

*H. pylori*

IL-8

6. Protein kinase protein phosphatase

Protein kinase protein phosphatase

*H. pylori*

IL-8

TNF IL-8

15 5 × 10<sup>5</sup>/ml

1 mM cycloheximide (Sigma), protein tyro-  
 sine kinase (PTK) 40 nM herbimycin A  
 (CalBiochem, San Diego, CA, U.S.A.), 250 µM  
 genistein (Sigma), protein kinase C (PKC)

1 µM staurosporin (Sigma), protein kinase A (PKA)  
 25 nM calyculin A (CalBiochem), pro-  
 tein phosphatase 10 µM H89 (CalBiochem)

108ml *H. pylori* 10 unit/ml

TNF 2 .

IL-8 ELISA

IL-8 mRNA RT-

PCR .

7. TNF

*H. pylori*

IL-8

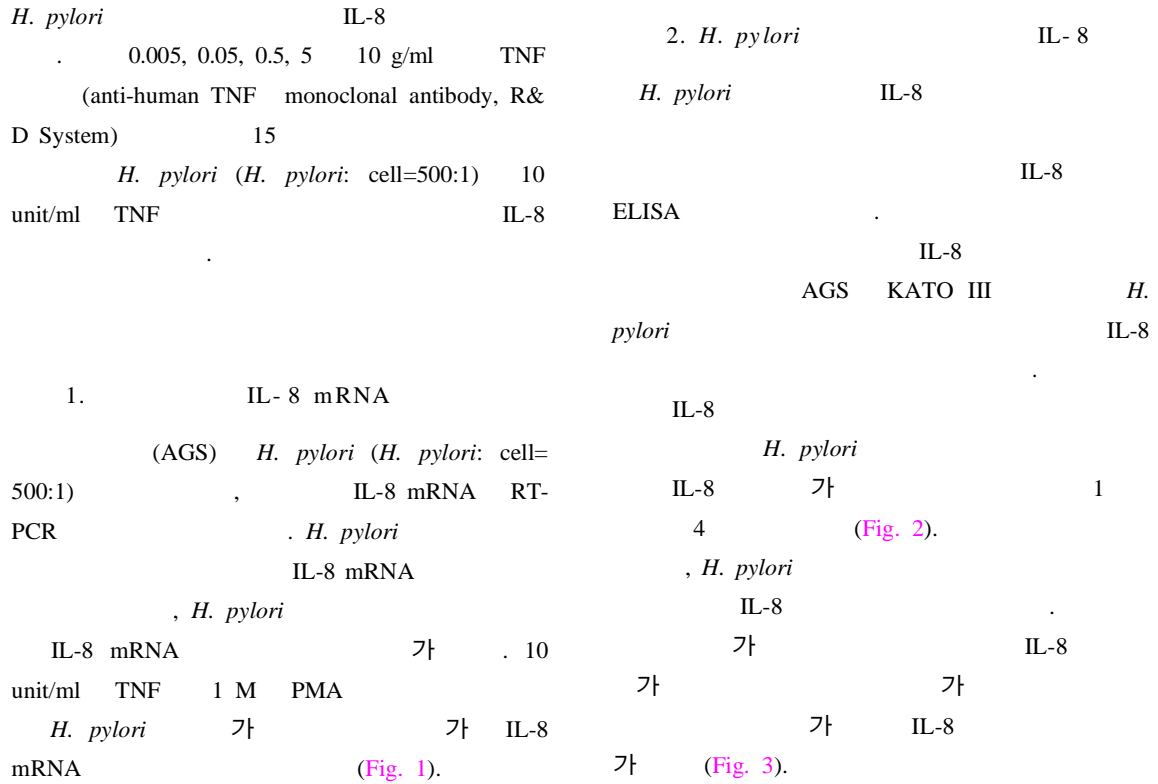
TNF

IL-8

가

가

TNF



**Fig. 1.** IL-8 mRNA expression by AGS cell line induced by various stimuli. Cellular RNA was extracted by guanidinium thiocyanate-phenol-chloroform method. IL-8 mRNA was reverse transcribed and then amplified. The densitometric ratios of IL-8 transcripts to  $\beta$ -actin transcripts upon control, TNF, *H. pylori* and PMA were 0.7, 1.44, 2.99 and 1.18, respectively. TNF 10 unit/ml, PMA 10 M.

**Fig. 2.** Kinetics of *H. pylori*-induced IL-8 production by AGS and KATO III cell lines stimulated with *H. pylori*. IL-8 produced in the culture supernatant was measured by ELISA.

**Fig. 3.** Effects of *H. pylori* to cell ratios on IL-8 production by AGS and KATO III cell lines. The number on X-axis represents the *H. pylori* to epithelial cell ratio. IL-8 from the culture supernatant was measured by ELISA. The incubation time was two hours.

3. TNF IL-8  
 IL-8 TNF KATO  
 IL-8  
 III가 AGS TNF 가  
 IL-8 가  
 가 (Fig. 4).

4. *H. pylori* 가 IL-8  
*H. pylori* 가  
 IL-8 H.  
*pylori* 가  
 . 0.5% formaldehyde H.  
*pylori* 가 4 IL-8  
 95% 100 10  
 4  
 IL-8  
 (Fig. 5).  
*H. pylori* IL-8  
 0.4 μm pore  
 cell culture insert  
 2  
 13.8-28.0% IL-8  
*H. pylori*  
 가 IL-8  
 (Fig. 5).

5. Protein kinase protein phosphatase  
 1) *H. pylori* IL-8 mRNA  
*H. pylori* IL-8 mRNA  
 protein kinase phosphatase  
 40 nM herbimycin A,  
 250 μM genistein 1 μM staurosporin  
 15 *H. pylori*  
 IL-8 mRNA . PKC stau-  
 rosporin PTK herbimycin A genis-  
 tein IL-8 mRNA  
 . IL-8 mRNA -  
 actin stauro-  
 sporin 0.11 가  
 herbimycin A genistein 0.38 1.02  
 IL-8 mRNA (Fig.  
 6). KATO III AGS IL-8 mRNA

**Fig. 4.** Effects of TNF on IL-8 production by AGS (A) and KATO III (B) cell lines. IL-8 produced in the culture supernatant of epithelial cells was measured by ELISA. The incubation time was two hours.

**Fig. 5.** Effects of various physical and chemical treatments of *H. pylori* on *H. pylori*-induced IL-8 production. Values represent percent production of IL-8 compared to non-treated *H. pylori*. The incubation time was three hours. Values represent the mean of paired samples among triplicate experiments.

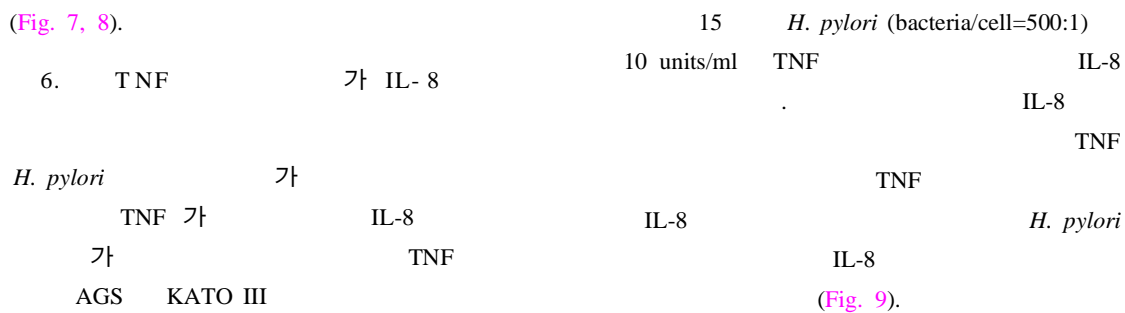
				PKC	staurosporin	PTK
						IL-8
2) <i>H. pylori</i>		IL-8			PKA	H89
<i>H. pylori</i> 7†	15		1	protein phosphatase	calyculin A	
mM cycloheximide, 40 nM herbimycin A, 250 μM				<i>H. pylori</i>	IL-8	
genistein, 1 μM staurosporin, 25 nm calyculin A, 10						
μM H89		IL-8		AGS KATO III		
	Cycloheximide	1 mM		(Fig. 7).		
95%						
genistein herbimycin A		PTK				
<i>pylori</i>		IL-8	<i>H.</i>	3) TNF	IL-8	
				TNF	IL-8	protein

**Fig. 6.** Effects of signal inhibitors on *H. pylori*-induced IL-8 mRNA expression of AGS cell line. The incubation time was one hour. Herbimycin A (40 nM), genistein (250 μM) and staurosporin (1 μM) were added 15 minutes before the stimulation with *H. pylori*. The densitometric ratios of IL-8 transcripts to β-actin transcripts with control, herbimycin A, genistein, staurosporin were 2.99, 0.38, 1.02 and 0.11 respectively.

**Fig. 7.** Percent inhibition by *H. pylori*-induced IL-8 production by AGS and KATO III cell lines pretreated with various inhibitors of signal pathway. IL-8 was measured from the culture supernatant 2 hours after the stimulation.

kinase	protein phosphatase		calyculin A	PKA	H89	
<i>H. pylori</i>			IL-8			(Fig. 8).
PKC	staurosporin	PTK	herbi-	<i>H. pylori</i>		IL-8
mycin A	genistein		IL-8	TNF	PKC	PTK
		protein phosphatase				





**Fig. 8.** Percent inhibition of TNF-induced IL-8 production by AGS and KATO III cell lines pretreated with various inhibitors of signal pathway. IL-8 was measured from the culture supernatant 2 hours after the stimulation. *H. pylori*:cell=500:1.

**Fig. 9.** Effects of anti-TNF antibody on IL-8 production. Culture media was pretreated with various doses of anti-TNF antibody 15 minutes before the stimulation with *H. pylori* or TNF 10 unit/ml. IL-8 was measured two hours after the stimulation.

*H. pylori* 가 IL-8

*H. pylori* B . *H. pylori*가

*H. pylori* (microvilli)가 (cytoskeletal system) (adhesion pedestal)

23 *H. pylori*가 가 가

가 *H. pylori* 45 .267 Enteropathogenic *Escherichia coli* (EPEC) 28 actin

IL-8 가 .621 , *H. pylori* TNF , IL-1, IL-6, inositol triphosphate가 *H. pylori* EPCE 14

*H. pylori* 가 *H. pylori* IL-8 *H. pylori*

.22 *H. pylori* IL-8 IL-1, IL-6 TNF 가 .810 *H. pylori* IL-8 ELISA *H. pylori* IL-8 *H. pylori* IL-8

*H. pylori* 가 (vacuolating cytotoxin; VacA), cytotoxin associated gene A (cagA), picA, picB, (urease), adhesin, flagella, heat shock protein, alcohol dehydrogenase ELISA IL-8 mRNA *H. pylori*

.5 , cagA *H. pylori*가 (constitutive expression) IL-8 TNF PMA IL-8 mRNA 가 cagA .23 cagA *H. pylori* 가 IL-8 mRNA 가 IL-8

가 IL-8 가 IL-8

가 .22 가 KATO III *H. pylori* IL-8 *H. pylori* IL-8

245 *H. pylori* TNF AGS IL-8 TNF *H. pylori*

IL-8 가 IL-8 IL-8  
 . 가 IL-8  
*H. pylori* *H. pylori*  
 LPS, (water extractable  
 surface protein), urease IL-8 protein  
 IL-1, TNF IL-8 kinase C가 ,29  
 .914 *H. pylori* IL-1 TNF  
 IL-8 tyrosine  
 IL-8 .30  
 100 *H. pylori*가  
 dehyde 0.5% formal- sine 가 145 kDa 105 kDa tyro  
 IL-8 tyrosine IL-8 27가 .  
 IL-8 tyrosin IL-8  
 . 4 IL-8  
 가 IL-8  
*H. pylori* , *H. pylori*  
 IL-8 가  
 0.4 m pore cell culture insert IL-8  
 IL-8 *H. pylori*  
*H. pylori* IL-8 가  
*H. pylori* IL-8  
*H. pylori* IL-8  
 가 protease elastase IL-8  
 18 . IL-8  
 IL-8 .27 *H. pylori*가  
 IL-8 .31 250 μM genistein  
 KATO III AGS  
 IL-8 . Herbimycin A

genistein 가 PTK src-tyrosine kinases 가 PKC  
 PKA 32 40  
 nM herbimycin A  
*H. pylori* IL-8 IL-8 가  
 genistein 가 PTK TNF IL-8  
 IL-8 TNF  
 staurosporin  
 Ca<sup>2+</sup>/calmodulin kinase, myosin light chain kinase, PKA, PKC, protein kinase G (PKG)  
 PTK *H. pylori* IL-8  
 가 33 staurosporin 25 nM IL-1, IL-6 TNF  
 1 *H. pylori*  
 μM 70-90% IL-8 가 TNF가  
 PKA H89 pro-IL-8 autocrine paracrine  
 tein phosphatase calyculin A 가  
 가 *H. pylori* IL-8 911 *H. pylori*  
 ( ) 가 IL-8 TNF RT-PCR  
 TNF IL-8 ( )  
 protein kinase protein phosphatase TNF TNF  
*H. pylori* *H. pylori*  
*H. pylori* PTK TNF TNF  
 가 IL-8 가 IL-8  
 PKC *H. pylori* IL-8  
 PKA protein phosphatase TNF 가  
 IL-8 *H.*  
 TNF IL-8 *pylori* IL-8 TNF  
*pylori* PTK PKC  
*H. pylori* IL-8  
 : *H. pylori*  
 PTK PKC *H. pylori*  
 27,29,30 protein phosphatase, PKA, interleukine-8  
 PKC, PTK IL-8  
 :  
 AGS KATO III *H. pylori*  
 ATCC 43504 *H. pylori* 가  
 , , ,  
 가 가

IL-8 ELISA RT-PCR

protein tyrosine kinase (PTK), protein kinase C (PKC), protein kinase A (PKA) protein phosphatase cycloheximide, herbimycin A, genistein, staurosporin, calyculin A H89

*H. pylori* IL-8

*H. pylori* 가 , ,

IL-8

*H. pylori* IL-8

PTK PKC PKA

protein phosphatase

TNF IL-8 *H.*

*pylori* PTK PKC

PKA protein phosphatase

가 . *H. pylori*

TNF *H. pylori*

IL-8

: *H. pylori*

IL-8 가

*H. pylori* IL-8

TNF IL-8 PTK

PKC가 TNF

: *Helicobacter pylori*, Interleukin-8, , protein kinase

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