PAPERS

Chemokines in the gastric mucosa in *Helicobacter pylori* infection

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Abstract

Background—Although chemokines have been suggested to play an important role in *Helicobacter pylori* associated gastritis, few studies have investigated the role of chemokines other than interleukin 8 (IL-8) in gastric mucosa.

Aims—To investigate the expression and production patterns of various chemokines using gastric biopsy specimens.

Methods—In 192 patients, expression patterns of C-X-C chemokines (IL-8 and growth regulated α (GRO α)) and C-C chemokines (regulated on activation, normal T cell expressed and presumably secreted (RANTES), monocyte chemotactic and activating factor (MCAF), macrophage inflammatory protein 1 α (MIP-1 α), and MIP-1 β) were examined using reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assay (ELISA). *cagA* gene was identified using PCR.

Results—*H pylori* infection was associated with increased rates of expression of mRNA for IL-8, GRO α , RANTES, and MIP-1 α and with increased levels of mucosal IL-8 and GRO α . IL-8 and GRO α levels correlated with the density of *H pylori* in both the antrum and corpus. The levels of these chemokines correlated with cellular infiltration in the antrum but not the corpus. *cagA* gene positive *H pylori* infection was associated with increased rates of expression of mRNA for IL-8 and GRO α and with increased levels of these chemokines.

Conclusion—*H pylori* infection is associated with increased expression rates and production of C-X-C chemokines (IL-8 and GRO α), but not with increased production of C-C chemokines. Although *H pylori* infection is associated with increased C-X-C chemokines in the antrum and corpus, there is a difference in the inflammatory response between these two areas of the stomach.

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Helicobacter pylori is a Gram negative bacterium thought to cause chronic gastritis, peptic ulcer,^{1,2} and gastric cancer.^{3,4} *H pylori* associated gastritis is fundamentally a bacterial infection of the gastroduodenal mucosal surface and as such is characterised by mucosal infiltration of polymorphonuclear leucocytes (PMNs) and mononuclear cells (MNCs).⁵ Cure of the infection results in healing of the gastritis and notable reduction in mucosal inflammation. The migration and activation of inflammatory cells into the mucosa is thought to be related to expression of various cytokines.⁶⁻¹⁵ A number of studies have investigated interleukin 8 (IL-8), which is known to be a neutrophil chemotactic factor,^{16,17} and have shown an association between levels of *H pylori* infection and IL-8 mRNA^{11,12,14} or IL-8 protein.^{7-10,13,15}

Cytokines showing leucocyte chemotactic activities, including IL-8, are referred to as chemokines.^{18,19} More than 20 members of the chemokine superfamily have been identified and shown to be involved directly or indirectly in inflammatory responses. Chemokines are classified into two major families on the basis of the arrangement of the first two of four conserved cysteine residues. The C-X-C chemokines consist of those members whose first two cysteines (C) are separated by one amino acid (X), while in the C-C chemokines the first two cysteines are adjacent to one another. In general, C-X-C chemokines mainly show chemotactic activities for neutrophils but not monocytes, whereas C-C chemokines show chemotactic activities for monocytes and lymphocytes but have little effect on neutrophils.²⁰ The former group includes IL-8 and growth regulated (GRO), and the latter group includes regulated on activation, normal T cell expressed and presumably secreted (RANTES), monocyte chemotactic and activating factor (MCAF), macrophage inflammatory protein 1 α (MIP-1 α), and MIP-1 β . Although IL-8 is closely associated with *H pylori* infection, few studies have investigated the relation between *H pylori* infection and chemokines other than IL-8.

To begin to clarify the relation between *H pylori* infection and chemokines, we examined chemokine expression and production patterns in gastric mucosa by reverse transcription polymerase chain reaction (RT-PCR) and enzyme linked immunosorbent assay (ELISA)

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using gastric biopsy specimens. In general, gastric mucosal inflammation caused by *H pylori* is more severe in the antrum than that in the corpus.^{21, 22} We therefore investigated the expression of chemokines in the antrum and corpus in patients with and without *H pylori* infection.

Materials and methods

PATIENT POPULATION

A total of 192 patients (110 men and 82 women; age range 21–81 years, mean 54.7 years) were studied; 172 were consecutive symptomatic adults who underwent endoscopy between October and December 1995. In order to examine the relation between *H pylori* and gastric cancer, 25 patients with gastric cancer, who underwent endoscopy between January and May 1996, also participated in this study. No patient had received non-steroidal anti-inflammatory drugs, proton pump inhibitors, or antibiotics within the previous three months. There was no overlap between subjects in this and in our previous studies. Endoscopic findings included: normal mucosa, 10 patients; gastritis without ulcer, 86 patients; gastric ulcer, 41 patients; duodenal ulcer, 30 patients; and gastric cancer, 25 patients. Ninety four patients were smokers. Informed consent was obtained from all patients and the protocol was approved by the ethics committee of Kyoto Prefectural University of Medicine.

Four biopsy specimens were taken from both the antrum (pyloric gland area) and corpus (fundic gland area); two were used for histological examination, one for *H pylori* culture and cytokine protein measurement, and one for cytokine mRNA measurement. Biopsy specimens were taken at least 2 cm from ulcers or tumours in the cases of gastric ulcer and gastric cancer.

The presence of *H pylori* was determined by culture and histological examination. Patients were classified as *H pylori* positive if at least one of the examinations gave a positive result. Some of the *H pylori* positive patients who had gastric ulcer or duodenal ulcer were treated with omeprazole 20 mg once daily, amoxicillin 2000 mg twice daily, and clarithromycin 400 mg twice daily, for two weeks. Four weeks after cessation of treatment, the patients were examined endoscopically again and biopsy specimens were taken as before. ¹³C urea breath test was also performed, and successful eradication of *H pylori* was determined if all test results were negative.

HISTOLOGY

The biopsy specimens were fixed in 10% buffered formalin, oriented on edge, embedded in paraffin wax, cut in sequential 4 µm sections, and stained with haematoxylin and eosin (H&E) and modified Giemsa stains. We usually cut three to five serial sections for one specimen and multiple high power fields were examined. Virtually all specimens included surface epithelium and muscularis mucosa. Specimens were examined by one experienced histologist blinded to the patient's clinical diagnosis or the characteristics of the *H pylori*

strain. The following features were evaluated: MNC infiltration, PMN infiltration, and density of *H pylori*. All variables were graded using the visual analogue scale graded from 0 to 3 for biopsy specimens from each site according to the Updated Sydney system.²³ For analysing the relation between the histological findings and the expression patterns of chemokines, we used the biopsy specimens from a mucosal site adjacent to the site where the biopsy specimens for chemokine measurements were obtained. MNC infiltration was scored as follows: 0 = occasional lymphocytes and plasma cells at a level considered normal; 1 = mild focal increase in MNC numbers; 2 = definite infiltrate with MNCs isolated from each other; and 3 = diffuse MNC infiltrate. PMN infiltration was scored as follows: 0 = no neutrophils; 1 = infiltrate only in the lamina; 2 = infiltrate within the epithelium; and 3 = infiltrate within the foveolar lumen. The density of *H pylori* was scored based on the average density on the surface and the foveolar epithelium. If areas with widely different scores were present on the same specimen, an average based on the general evaluation of the sample was used by the observer. If intestinal metaplasia was present, only areas without metaplasia were evaluated for the presence of *H pylori*.

H PYLORI CULTURE

Biopsy specimens were placed in 1.5 ml of phosphate buffered saline (PBS; pH 7.4), and were immediately homogenised using a tissue homogeniser (Kontes, Vineland, New Jersey, USA). Ten ml aliquots of homogenate supernatant were spread on Skirrow agar medium containing 8% horse blood, vancomycin (10 mg/ml), polymyxin B (2.5 U/ml), and trimethoprim (5 mg/ml) and incubated at 37°C under microaerophilic conditions (AnaeroPack Systems; Mitsubishi Gas Chemical Co., Inc., Osaka, Japan) for up to five days. The organisms were identified as *H pylori* by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions. The strains were cultured again at 37°C under microaerophilic conditions in brain heart infusion broth (Nissui Co., Ltd., Osaka, Japan) containing 5% horse serum for 24 to 48 hours on a shaker, and aliquots were stored at –80°C in brain heart infusion broth containing 15% horse serum and 15% glycerol.

PREPARATION OF GNOMIC DNA FROM CLINICAL ISOLATES

The culture medium was centrifuged for five minutes at 10 000 g and the bacterial pellets were washed three times with PBS (pH 7.4). The pellets were each suspended in 200 ml of distilled water and were boiled for 10 minutes. The supernatants, obtained by centrifugation (10 000 g for five minutes), were stored at –20°C until use as PCR templates.

PREPARATION OF RNA AND cDNA FOR CHEMOKINE mRNA MEASUREMENT

The biopsy specimens were immediately placed in guanidine isothiocyanate buffer, frozen on dry ice, and stored at –80°C until

use. Samples were homogenised using a tissue homogeniser (Kontes), and total RNA was extracted by the acid guanidinium isothiocyanate-phenol-chloroform method as described previously.²⁴ Aliquots (1 mg) of total RNA were incubated at 65°C for five minutes, chilled on ice, and reverse transcribed in a final volume of 10 ml of a solution containing 50 mmol/l Tris-HCl (pH 8.3), 75 mmol/l KCl, 3 mmol/l MgCl₂, 10 mmol/l dithiothreitol, 200 mmol/l each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate (Pharmacia Biotech AB, Uppsala, Sweden), 1 mmol/l oligo(dT)16 primer, 20 units RNasin (Ribonuclease inhibitor; Toyobo Co., Ltd, Tokyo, Japan), and 100 units of MoMuLV RNase H⁻ reverse transcriptase (BRL Co., Gaithersburg, Maryland, USA). The mixture was incubated at 43°C for 90 minutes, heated to 95°C for 10 minutes, and stored at -20°C until use.

POLYMERASE CHAIN REACTION

Bacterial DNA and cDNA from biopsy specimens were denatured by heating to 95°C for 10 minutes and cooled on ice for five minutes. Bacterial DNA and cDNA (5 ml) were added to 50 ml reaction mixtures containing 5 ml 10× PCR reaction buffer (500 mmol/l KCl, 100 mmol/l Tris-HCl (pH 8.8), 15 mmol/l MgCl₂, 1% Triton X-100, 200 mmol/l each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate, 200 nmol/l of each primer, and 1.0 unit Taq DNA polymerase (Boehringer, Mannheim, Germany), and H₂O). Amplification was performed using a Takara PCR Thermal Cycler 480 (Takara Biomedicals, Otsu, Japan) for 35 cycles (for chemokine PCRs) or 30 cycles (for 16S rRNA and *cagA* PCRs), each of which consisted of one minute at 95°C for denaturation, one minute at 60°C (for chemokine PCRs) or 50°C (for 16S rRNA and *cagA* PCRs) for annealing, and one minute at 72°C for extension. The final cycle included an extension step for seven minutes at 72°C to ensure full extension of the product. Aliquots (10 ml) of each PCR product were analysed by electrophoresis on 1.5% agarose (Dojin Chemical Co., Ltd, Kyoto, Japan) gels containing ethidium bromide, and the bands were examined under ultraviolet light for the presence of the amplified DNA.

Oligonucleotide primers were designed based on previous studies.^{15 25-28} The sense and

antisense primers specific for each chemokine were designed to include at least one intron, permitting distinction between amplified cDNA and possible contaminating residual genomic DNA. Table 1 shows chemokine specific oligonucleotide primers. β-Actin and *H. pylori* 16S rRNA were assayed as positive controls for chemokine measurement and for the presence of *H. pylori*, respectively. In addition, *cagA* gene negative strains, G21, G50, and 2U (kindly provided by Dr J E Crabtree), were simultaneously assayed to check for contamination.

CHEMOKINE PROTEIN MEASUREMENT

For chemokine protein measurement, we used the same biopsy specimens as used for *H. pylori* culture. Biopsy specimens were placed in 1.5 ml of PBS (pH 7.4), and were immediately homogenised using a tissue homogeniser as described earlier. Aliquots of homogenate supernatants, obtained by centrifugation (10 000 *g* for 10 minutes), were stored at -80°C until assay for total proteins by a modified Lowry method and for chemokine protein by ELISA. IL-8, GROα, MIP-1α, and RANTES in biopsy homogenate supernatants were measured by ELISA using commercially available assay kits (Research and Diagnostic Systems, Minneapolis, Minnesota, USA). Assays were performed in duplicate according to the manufacturer's instructions. In our laboratory, the ELISA sensitivities of IL-8, GROα, MIP-1α, and RANTES were approximately 10 pg/ml.

STATISTICS

The mucosal levels of IL-8, GROα, MIP-1α, and RANTES protein were expressed as pg/mg biopsy protein, and data were expressed as median and range. As histological scores are non-parametric, these scores are presented as median rather than mean values. The two tailed Fisher's exact probability test was used to compare RT-PCR results between patient populations. This test was also used to compare differences of chemokine mRNA before and after treatment. Wilcoxon's matched pairs test was used to compare differences of chemokine protein before and after treatment. The relation between histological findings and the production of chemokines was assessed by Spearman's rank correlation coefficient. This test was also used to assess the relation between the production of IL-8 and GROα. For the remaining statistical analyses, the Mann-Whitney U test for non-parametric data was used. A *p* value of less than 0.05 was accepted as statistically significant.

Results

H. PYLORI INFECTION AND ENDOSCOPIC AND HISTOLOGICAL FINDINGS

Of the 192 patients examined, 131 (68.2%) were positive for *H. pylori* (table 2). Of the 123 patients with positive culture for *H. pylori*, 114 (92.7%) were positive for the *cagA* gene.

As expected specimens from patients with endoscopic normal mucosa without *H. pylori* had no infiltration of MNCs or PMNs and specimens from those with *H. pylori* infection

Table 1 Human chemokine specific oligonucleotide primers for RT-PCR

Chemokine	Primer	Nucleotide	cDNA (bp)
IL-8	Sense	GCTTTCTGATGGAAGAGAGC	585
	Antisense	GGCACAGTGGACAAGGACT	
GROα	Sense	TTGCAGACCCTGCAGGGAAT	184
	Antisense	TGGATTGTCACTGTTTCAGC	
RANTES	Sense	TGCCTCCCATATTCCTCGG	211
	Antisense	CTAGCTCATCTCCAAAGA	
MIP-1α	Sense	CCTTGCTGTCTCTCTCTGCA	254
	Antisense	CACTCAGCTCTAGGTCGCTG	
MIP-1β	Sense	TGTTCTCTCTCATGCTAGTA	233
	Antisense	GTACTCCTGGACCCAGGAT	
MCAF	Sense	CAATAGGAAGATCTCAGTGC	188
	Antisense	GTGTTCAGTCTTCGGAGTT	

Table 2 *H. pylori* infection and endoscopic findings

Endoscopic findings	<i>H. pylori</i> positive			<i>H. pylori</i> negative	<i>H. pylori</i> positive/total (%)	<i>cagA</i> +/(<i>cagA</i> + and <i>cagA</i> -) (%)
	<i>cagA</i> +	<i>cagA</i> -	Not determined*			
Chronic gastritis (n=86)	45	5	3	33	61.6	90.0
Gastric ulcer (n=41)	25	2	3	11	73.2	92.6
Duodenal ulcer (n=30)	28	1	1	0	100	96.6
Gastric cancer (n=25)	16	1	1	7	72.0	94.1
Normal mucosa (n=10)	0	0	0	10	0.0	—
Total (n=192)	114	9	8	61	68.2	92.7

*The presence of the *cagA* gene could not be detected as these patients were *H. pylori* positive on histology only.

showed typical changes with infiltration of PMNs and MNCs. There was no significant difference in density of *H. pylori* between the antrum and the corpus. In contrast, both MNC and PMN infiltration was significantly more severe in the antrum than in the corpus in *H. pylori* infection.

H. PYLORI INFECTION AND EXPRESSION OF CHEMOKINE mRNA

H. pylori infection was associated with increased rates of expression of mRNA for IL-8, GRO α , RANTES, and MIP-1 α in both the antrum and corpus (table 3). In contrast mRNA for MIP-1 β and MCAF was detected in the majority of specimens independent of whether *H. pylori* infection was present or absent.

To reduce the variability because of possible differences in histology related to different diseases, we examined whether expression of

these chemokine mRNAs was related to the histological findings in patients with only *H. pylori* gastritis (table 4). As was true for the overall population, in the antrum both MNC and PMN infiltration scores were significantly higher in specimens positive for the expression of IL-8 and GRO α mRNA. In the corpus, MNC infiltration score was similar in specimens positive or negative for expression of IL-8 or GRO α mRNA. When mRNA for both IL-8 and GRO α was expressed, the MNC and PMN infiltration scores were significantly higher in both the antrum and corpus (data not shown).

In both the antrum and corpus, the MNC infiltration score was significantly higher in specimens positive for the expression of MIP-1 α mRNA than in those negative for the expression of this chemokine mRNA. No relation was found in either the antrum or corpus between C-C chemokine mRNA and PMN

Table 3 Expression of chemokine mRNA and *H. pylori* infection

	No studied	C-X-C chemokine (%)		C-C chemokine (%)			
		IL-8	GRO α	RANTES	MIP-1 α	MIP-1 β	MCAF
Antrum							
<i>H. pylori</i> positive	131	79.4‡	77.1‡	57.3‡	71.8‡	84.0	95.4
<i>H. pylori</i> negative	40	2.5	0	25.0	12.5	82.5	87.5
Corpus							
<i>H. pylori</i> positive	131	71.8‡	76.3‡	54.2*	67.2‡	81.7	96.2
<i>H. pylori</i> negative	40	0	0	30.0	10.0	80.0	85.0

Chemokine expression in *H. pylori* positive specimens was compared with that in *H. pylori* negative specimens with normal histology. *p<0.01, †p<0.0005, ‡p<0.00001, two tailed Fisher's exact probability test.

Table 4 Relation between histological findings and chemokine profiles in *H. pylori* positive patients with chronic gastritis

	C-X-C chemokine				C-C chemokine					
	IL-8		GRO α		RANTES		MIP-1 α		MIP-1 β	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
No studied										
Antrum	39	11	40	10	27	23	38	12	40	10
Corpus	36	14	38	12	26	24	37	13	38	12
MNC infiltration score										
Antrum	2	1.5	2	1.5	2	2	2	1.5	2	2
	p<0.0001		p<0.0005		NS		p<0.005		NS	
Corpus	2	2	2	2	2	2	2	1.5	2	2
	NS		NS		NS		p<0.001		NS	
PMN infiltration score										
Antrum	2	0.5	2	0.5	2	2	2	2	2	2
	p<0.0001		p<0.0005		NS		NS		NS	
Corpus	1	0.5	1	1	1	1	1	1	1	1
	p<0.05		NS		NS		NS		NS	
<i>H. pylori</i> density score										
Antrum	2	1	2	1	2	2	2	1	2	1.5
	p<0.01		p<0.005		NS		p<0.0001		NS	
Corpus	2	1	2	1	2	2	2	1	2	2
	p<0.0005		p<0.01		NS		p<0.005		NS	

Positive, specimens positive for the expression of the corresponding chemokine mRNA; negative, specimens negative for the expression of the corresponding chemokine mRNA.

Data on MCAF mRNA are not shown as all *H. pylori* positive specimens with chronic gastritis expressed MCAF mRNA.

Histological variables were scored from 0 to 3; the median score is shown.

Significance was determined using the Mann-Whitney U test.

Figure 1 *H. pylori* infection and production of GROα. Bars indicate median values for each group.

infiltration. *H. pylori* density score was significantly higher in specimens positive for the expression of IL-8, GROα, or MIP-1α mRNA than in those negative for the expression of mRNA for these chemokines both in the antrum and corpus (table 4).

With regard to the relation between the expression of chemokine mRNA and the endoscopic findings in *H. pylori* positive specimens, the positive rate of expression of IL-8 mRNA was significantly higher in the antrum than that in the corpus in patients with duodenal ulcer (86.7% versus 56.7%, $p < 0.05$, $n = 30$) which is consistent with the association of duodenal ulcer with antral predominant gastritis.

H. PYLORI INFECTION AND PRODUCTION OF CHEMOKINES

mRNA levels of IL-8, GROα, RANTES, and MIP-1α were increased in *H. pylori* infection and the level of production of these chemokines was examined at the protein level by ELISA in 100 patients, 76 of whom were infected with *H. pylori*. There were no significant differences in sex or age distribution between these patients and the total patient population. The mucosal levels of IL-8 and GROα protein were significantly increased in *H. pylori* infection in the antrum and corpus (median for IL-8 was 58 pg/mg versus 0 pg/mg, and 60.6 pg/mg versus 0 pg/mg for antrum and corpus, respectively; $p < 0.0001$). For GROα the median was 157.3 pg/mg versus 0 pg/mg and 157.4 pg/mg versus 0 pg/mg for antrum and corpus, respectively ($p < 0.0001$; fig 1). In addition, the mucosal levels of IL-8 and GROα protein were significantly higher in specimens positive for expression of IL-8 and GROα mRNA compared with those in specimens without expression of these transcripts (median

for IL-8 was 84.5 pg/mg versus 0 pg/mg, and 88.5 pg/mg versus 0 pg/mg for antrum and corpus, respectively; $p < 0.0001$). For GROα the median was 183.1 pg/mg versus 0 pg/mg and 180 pg/mg versus 96.4 pg/mg for antrum and corpus, respectively ($p < 0.0001$). The mucosal level of GROα protein was significantly higher in cagA positive than in cagA negative specimens in the antrum (fig 1).

The mucosal level of IL-8 protein was significantly correlated with that of GROα protein both in the antrum and corpus ($p < 0.0001$ by Spearman rank test; fig 2). There was no relation between the level of mucosal RANTES protein between *H. pylori* infected and uninfected individuals (median 251.8 pg/mg versus 256.1 pg/mg and 286.4 pg/mg versus 293.3 pg/mg for antrum and corpus, respectively). In the antrum, the RANTES protein level was significantly higher in specimens positive for the expression of RANTES mRNA than in those negative by RANTES RT-PCR (median 282.3 pg/mg versus 228.8 pg/mg, respectively, $p < 0.05$). The mucosal level of MIP-1α protein was below the threshold of sensitivity in all specimens.

In the corpus, mucosal levels of IL-8 and GROα protein were significantly lower in patients with duodenal ulcer compared with those in patients with gastritis without ulcers (median for IL-8 of 10.1 pg/mg versus 90 pg/mg, and median for GROα of 102.4 pg/mg versus 167 pg/mg, for antrum and corpus respectively; $p < 0.05$). On the other hand, the mucosal levels of IL-8 protein were significantly higher in patients with duodenal ulcer compared with those in patients with gastritis without ulcers in the antrum (median 102.3 pg/mg versus 59.9 pg/mg; $p < 0.05$).

Figure 2 Relation between mucosal levels of IL-8 and GRO α protein in *H. pylori* positive patients.

We examined the relation between the production of chemokines and histological findings in *H. pylori* chronic gastritis. The mucosal levels of IL-8 and GRO α protein were significantly correlated with infiltration of MNCs and PMNs in the antrum (fig 3). There was no significant difference in the corpus. The mucosal levels of IL-8 and GRO α protein were significantly correlated with the density of *H. pylori* in both the antrum and corpus. *H. pylori* positive specimens with diseases other than chronic gastritis also showed a similar tendency (data not shown).

EXPRESSION AND PRODUCTION OF CHEMOKINES AND ERADICATION OF *H. PYLORI*

Twenty patients were treated with eradication therapy. Ten had gastric ulcer and 10 had duodenal ulcer; all patients were infected with *H. pylori* possessing the *cagA* gene. *H. pylori* infection was successfully cured in 15 patients, seven with gastric ulcer and eight with duodenal ulcer. The positive rates of expression of IL-8, GRO α , and MIP-1 α mRNA decreased significantly following successful treatment of patients (data not shown). There was no significant change in the expression of

Figure 3 Production of C-X-C chemokines and histological findings in *H. pylori* positive patients with chronic gastritis are expressed as box plots. The ends of the bars indicate the 25th and 75th percentiles. The 50th percentile is indicated with a line, and the 10th and 90th percentiles are indicated with error bars. Data outside the 10th and 90th percentile are displayed using symbols. Correlation coefficients were calculated with the Spearman rank test.

Figure 4 Production of GROα and eradication of *H. pylori*. * $p < 0.005$ by Wilcoxon's matched pairs test. Before, before eradication therapy; after, after eradication therapy.

RANTES, MIP-1β, and MCAF mRNA. The mucosal levels of IL-8 and GROα protein fell below the level of detection in all but one and two patients, respectively (fig 4). The mucosal level of RANTES protein was not reduced even in successfully treated patients.

EXPRESSION AND PRODUCTION OF CHEMOKINES AND THE *cagA* GENE

As expected, the positive rate of expression of IL-8 mRNA was significantly higher in *cagA* gene positive strains (*cagA*⁺ specimens) than in *cagA* gene negative strains (*cagA*⁻ specimens) in the antrum (86.8% versus 44.4%, $p < 0.005$). Similarly, the mucosal levels of IL-8 protein were also significantly increased in *cagA*⁺ compared with *cagA*⁻ specimens in both the antrum and corpus (median 65.3 pg/mg versus 0 pg/mg and 73.7 pg/mg versus 10 pg/mg for antrum and corpus, respectively; $p < 0.05$). The positive rate for expression of GROα mRNA was significantly higher in *cagA*⁺ than in *cagA*⁻ specimens in the corpus (83.3% versus 44.4%, $p < 0.05$). The mucosal level of GROα protein was significantly increased in *cagA*⁺ over that in *cagA*⁻ specimens in the antrum (median 173.2 pg/mg versus 83.0 pg/mg, $p < 0.001$; fig 1). Expression of C-C chemokine mRNA did not differ between *cagA*⁺ and *cagA*⁻ specimens in either the antrum or corpus.

Discussion

In the present study, *H. pylori* infection was associated with increased expression and production of not only the C-X-C chemokine IL-8 but also GROα. Furthermore, the expression of C-X-C chemokines significantly decreased in patients after successful eradication. These findings are consistent with the C-X-C chemokines playing important roles in *H. pylori* associated gastric inflammation. The degree of cellular infiltration was however lower in the corpus than that in the antrum but there were no differences in expression of C-X-C chemo-

kines between these areas, suggesting that other, as yet unidentified, factors modulate the mucosal response between C-X-C chemokine expression and cellular infiltration. It is of interest that the mucosal levels of C-X-C chemokines correlated significantly with the density of *H. pylori* in both the antrum and corpus, whereas the mucosal levels of C-X-C chemokines were significantly correlated with cellular infiltration only in the antrum. However, even in the corpus, expression of both IL-8 and GROα mRNA was associated with significantly more severe MNC and PMN infiltration, suggesting that IL-8 and GROα may act cooperatively and that their synergistic action causes severe inflammation even in the corpus with low responsiveness to either chemokine alone. There are two types of IL-8 receptor: type I is IL-8 specific and type II has affinity for GROα.²⁹ Further studies will be necessary to investigate whether the numbers of these receptors differ between the antrum and corpus and whether such a difference might be related to the differences in inflammatory response to *H. pylori* between these two areas of the stomach.

With regard to C-C chemokines, the positive rate of expression of MIP-1α mRNA was significantly enhanced in *H. pylori* positive specimens. Furthermore, the positive rate of expression of MIP-1α mRNA significantly decreased after successful eradication. The MNC infiltration score was significantly higher in specimens positive for the expression of MIP-1α mRNA compared with those negative for its expression in both the antrum and corpus. Expression of MIP-1α mRNA was not associated with PMN infiltration. These data are in agreement with previous observations that MIP-1α has chemotactic activity for MNCs but has little effect on PMNs.²⁰ However, as production of MIP-1α protein was below the threshold of sensitivity in all patients, the role of this chemokine cannot be determined from this study.

While the positive rate of expression of RANTES mRNA was significantly higher in *H pylori* positive infection, there were no differences in protein levels. The mucosal level of RANTES protein was significantly higher in specimens positive for the expression of RANTES mRNA than in those negative for the expression of this mRNA in the antrum. Similar conflicting data have been reported for some other cytokines including IL-1 α ,¹³ IL-7,^{14,15} and IL-10.^{14,15} These conflicts between protein and mRNA levels are possibly related to disturbances in the pathway from mRNA to protein expression, degradation of mRNA prior to translation into protein, or the fact that the amount of mRNA may not exceed the threshold required for protein production.

We found no correlation between RANTES mRNA and protein expression; the degree of MNC and PMN infiltration and the levels of expression of RANTES were not decreased after successful eradication therapy. These findings suggest that RANTES may not play a role in MNC or PMN infiltration in *H pylori* infection. RANTES does not show any chemotactic activity on PMNs,³⁰ but shows chemotactic activity on T lymphocytes³¹ and eosinophils.^{30,32} Further studies will be necessary to investigate the relation between infiltration by these cells and RANTES expression in gastric mucosa.

MIP-1 β and MCAF mRNA were detected in the majority of specimens regardless of the presence or absence of *H pylori*. It has been thought that chemokines are not expressed in normal cells, but as the stomach is constantly exposed to various stimuli including dietary components, these influences may induce expression of chemokine mRNA.

In the antrum, the mucosal levels of IL-8 and GRO α protein, the positive rate of expression of IL-8 mRNA, and *H pylori* density were all significantly higher in patients with duodenal ulcer than in those with other diseases. In contrast, in the corpus, the mucosal levels of IL-8 protein and *H pylori* density were significantly lower in patients with duodenal ulcer than in those with other diseases. It is not clear what relation may exist between these observations and the formation of duodenal ulcer disease in this study.

Finally, we examined the *cagA* gene. Recent studies using *cagA* isogenic mutant strains indicated that the *cagA* gene was not essential for induction of IL-8, but was a phenotypic marker for *H pylori* strains capable of inducing IL-8.^{33,34} Genes coexpressed with the *cagA* gene, present in the upstream sequence of the *cagA* gene, are thought to contribute to IL-8 induction.^{34,35} Recently, a region of approximately 40 kilobases responsible for virulence that contains the *cagA* genes was reported and is referred to as the "cag pathogenicity island".³⁶ *cagA* positive strains are, therefore, regarded as strains possessing the cag pathogenicity island. In agreement with previous studies,¹³⁻¹⁵ expression and production of IL-8 were enhanced by *cagA* positive strains compared with *cagA* negative strains. In this study the positive rate of expression of GRO α mRNA in the corpus and the mucosal level of GRO α

protein in the antrum was also significantly higher in infection with *cagA* positive *H pylori*. There was no association between C-C chemokines and the *cagA* gene.

In conclusion, *H pylori* infection is associated with increased expression and production of C-X-C chemokines IL-8 and GRO α , but not C-C chemokines. Although *H pylori* induces C-X-C chemokines both in the antrum and corpus, the association between chemokine expression and inflammation is less in the corpus.

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