Increased gastric production of interleukin-8 and tumour necrosis factor in patients with *Helicobacter pylori* infection

X-G Fan, A Chua, X-J Fan, P W N Keeling

Abstract

***Aims*—To investigate the role of interleukin-8 (IL-8) and tumour necrosis factor (TNF) in patients infected with *Helicobacter pylori*.**

***Methods*—The study population comprised 52 patients with dyspepsia attending for upper gastrointestinal endoscopy. Of these patients, 35 were infected with *H pylori*. IL-8 and TNF concentrations in plasma, gastric juice, and gastric biopsy homogenate supernatant fluid were measured by radioimmunoassay and L929 cell bioassay, respectively.**

***Results*—The concentrations of IL-8 and TNF in gastric juice and gastric biopsy homogenates were substantially greater in patients infected with *H pylori*. In *H pylori* positive patients IL-8 concentrations in gastric juice and gastric biopsy homogenates were higher in those with moderate gastritis than in those with mild gastritis. There was a positive correlation between IL-8 and TNF concentrations in gastric juice and gastric biopsy homogenate supernatant fluid from *H pylori* positive patients. There were no significant differences between *H pylori* positive and negative patients with respect to IL-8 and TNF plasma concentrations.**

***Conclusion*—This study suggests that increased gastric production of IL-8 and TNF may be implicated in the pathogenesis of *H pylori* associated gastroduodenal disease.**

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Keywords: Interleukin-8, tumour necrosis factor, *Helicobacter pylori*.

Helicobacter pylori infection has been implicated in the pathogenesis of active chronic gastritis and peptic ulcer disease. Neutrophil accumulation in gastric tissue is one of the characteristics of this condition.[1 2] Inflammatory mediators, such as interferon-γ, have been closely correlated with *H pylori* associated gastritis and duodenal ulcer.[3 4] Little is known, however, about the potential importance of interleukin-8 (IL-8), a novel cytokine which activates neutrophils, in patients infected with *H pylori*.

Interleukin-8 is a potential mediator of the inflammatory response. In addition to chemotactic potential, IL-8 is capable of activating polymorphonuclear leucocyte degranulation, the respiratory burst, and the 5-lipoxygenase pathway.[5 6] Interleukin-8 is generated by a variety of immune and non-immune cells, including macrophages/monocytes, endothelial cells, fibroblasts, hepatocytes, and polymorphonuclear leucocytes[5 7–9]; IL-8 may also be a component of the inflammatory cascade. Tumour necrosis factor (TNF) has many important biological functions and is produced mainly by activated macrophages/monocytes and T lymphocytes.[10] In addition to its oncolytic activity, it may be a primary mediator in the pathogenesis of infection, injury, and inflammation.[11 12] As such, TNF may exert an effect on IL-8, a hypothesis tested in this study.

Methods

Prepyloric antral biopsy specimens were obtained from 52 patients with dyspepsia attending for upper gastrointestinal endoscopy. None of the patients studied were taking non-steroidal anti-inflammatory drugs, bismuth compounds, or antibiotics. Patients with evidence of malignant disease and those undergoing immunosuppressive treatment were excluded. *H pylori* infection was assessed histologically by Giemsa stain and the rapid urease test (CLO-test, Delta West Ltd., Bentley, Australia). Of the 52 patients, 35 (19 men and 16 women; mean age 45·6 years, range 20–73 years) were infected with *H pylori*, 14 of whom had duodenal ulcer, while a further 21 had gastritis. The remaining 17 patients (seven men and 10 women; mean age 44·1 years, range 22–77 years) were *H pylori* negative with normal results on gastrointestinal endoscopy and histology. These patients were diagnosed as having functional dyspepsia.

Plasma was isolated from venous blood with heparin as the anti-coagulant. Gastric juice was collected by aspiration of patients during endoscopy following a 12 hour fast, and was then centrifuged immediately at 3000 rpm at 4°C for 30 minutes. Gastric biopsy specimens were immediately washed with phosphate buffered saline (PBS; pH 7·4), weighed, placed in a tube containing 0·5 ml 0·01 M PBS, and gently ground with a glass homogeniser for three minutes at 4°C. Biopsy homogenates were then centrifuged at 3000 rpm at 4°C for 30 minutes and the supernatant fluid removed.[13] All samples were stored at −70°C until analysed. Sample collection was not always successful and thus samples were not obtained from all patients or were sometimes too small to enable assay of both IL-8 and TNF.

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Interleukin-8 was measured by radioimmunoassay (Advanced Magnetics Inc., Cambridge, Massachusetts, USA). The assay was performed in duplicate according to the manufacturer's instructions. Tumour necrosis factor bioactivity was measured by bioassay of in vitro cytotoxicity against L929 cells. These assays have been evaluated previously.[14,15] Briefly, L929 cells were incubated for 24 hours at 37°C in 96-well flat-bottomed microtitre plates at a concentration of 4×10^3 cells/well. Titrations of the TNF standard (Sigma, Lab Supplies Ltd., Dublin, Ireland) and dilutions of the samples in medium containing 2 μg/ml actinomycin D (Sigma) were added to the wells and incubated for 24 hours at 37°C. Then 20 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma), at a concentration of 6 mg/ml, were added to the wells and the plates incubated for four hours at 37°C. MTT was then removed and 200 μl of dimethyl sulphoxide (DMSO) added to each well. Absorbance was read at 570 nm.[16]

Data are expressed as median (range) and were analysed using the Mann–Whitney U test for non-parametric data and linear regression analysis.

Table 1 Interleukin-8 concentrations in H pylori positive and negative patients

Patient status	*Plasma (ng/ml)*	*Gastric juice (ng/ml)*	*Gastric biopsy homogenates (ng/g wet weight)*
H pylori positive			
median	0·090	32·60	8·86
range	0·002–0·215	5·04–39·47	3·90–20·09
no. of patients	34	31	35
H pylori negative			
median	0·110	21·37**	6·31*
range	0·003–0·186	0·730–31·35	3·16–14·81
no. of patients	15	17	17

* p<0·05, ** p<0·01 compared with *H pylori* positive subjects.

Table 2 Interleukin-8 concentrations in H pylori positive patients with gastritis

Patient status	*Plasma (ng/ml)*	*Gastric juice (ng/ml)*	*Gastric biopsy homogenates (ng/g wet weight)*
Mild gastritis			
median	0·10	20·25	8·50
range	0·002–0·215	6·50–36·39	3·90–12·24
no. of patients	10	10	10
Moderate gastritis			
median	0·103	33·50*	11·38*
range	0·003–0·194	5·04–39·47	5·31–20·09
no. of patients	11	10	11

* p<0·05 compared with patients with mild gastritis.

Table 3 Interleukin-8 concentrations in H pylori positive patients with duodenal ulcer or gastritis alone

Patient status	*Plasma (ng/ml)*	*Gastric juice (ng/ml)*	*Gastric biopsy homogenates (ng/g wet weight)*
Duodenal ulcer			
median	0·092	35·0	9·0
range	0·008–0·143	11·22–37·12	4·92–12·11
no. of patients	13	11	14
Gastritis			
median	0·103	31·0	10·25
range	0·002–0·215	5·04–39·4	3·90–20·09
no. of patients	21	20	21

Table 4 Tumour necrosis factor concentrations in H pylori positive and negative patients

Patient status	*Plasma (ng/ml)*	*Gastric juice (ng/ml)*	*Gastric biopsy homogenates (ng/g wet weight)*
H pylori negative			
median	19·0	213·75	101·0
range	10·60–50·48	100·51–417·89	41·56–127·62
no. of patients	12	12	12
H pylori positive			
median	22·12	313·75*	173·33**
range	11·86–63·46	104·89–542·61	69·90–311·24
no. of patients	27	27	27

* p<0·05, ** p<0·001 compared with *H pylori* negative patients.

Results

Interleukin-8 concentrations in gastric juice and biopsy homogenate supernatant fluid were increased in patients with *H pylori* infection compared with those without *H pylori* infection, but this difference was not observed for plasma IL-8 concentrations (table 1). Interleukin-8 concentrations in gastric juice and biopsy homogenate supernatant fluid were increased in patients with moderate compared with those with mild gastritis; there was no difference in plasma IL-8 concentrations between these two groups (table 2). Furthermore, IL-8 concentrations in plasma, gastric juice and biopsy

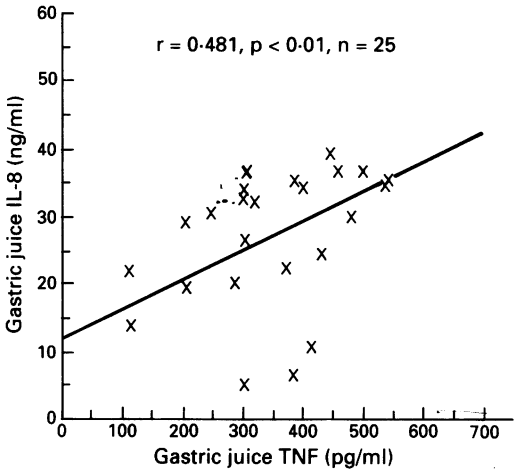

Figure 1 Correlation between IL-8 and TNF concentrations in gastric juice of H pylori positive patients.

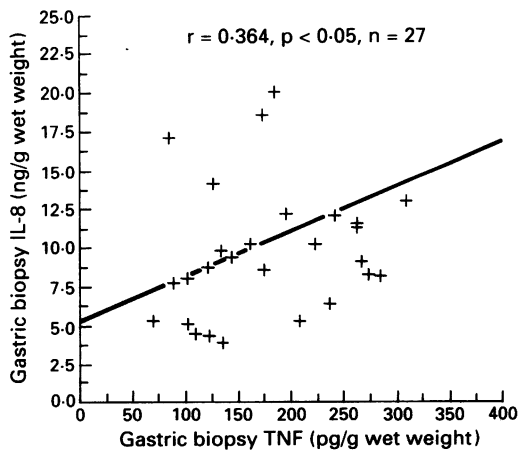

Figure 2 Correlation between IL-8 and TNF concentrations in gastric biopsy homogenate supernatant fluid of H pylori positive patients.

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homogenate supernatant fluid were not significantly different in patients with duodenal ulcer when compared with those with gastritis alone (table 3).

Tumour necrosis factor concentrations in gastric juice and biopsy homogenate supernatant fluid were significantly higher in *H pylori* positive patients (table 4), but were not significantly different ($p>0.05$) when patients with gastritis were compared with those with duodenal ulcer or when patients with moderate gastritis were compared with those with mild gastritis (data not shown).

There was a positive correlation between IL-8 and TNF concentrations in gastric juice (fig 1) and in gastric biopsy homogenate supernatant fluid (fig 2) of *H pylori* positive patients.

To investigate whether TNF from gastric juice is affected by pH, the following experiment was performed: dilutions (50 pg/ml) of TNF standard were added to six tubes and pH values were adjusted to 3, 7·4, and 8·5, in duplicate, using HCl or NaOH, respectively. The concentration of TNF in each of these tubes was measured. The results (48·22, 50·54, and 48·70 pg/ml TNF, respectively) indicated that pH has no apparent influence on TNF activity. As the IL-8 radioimmunoassay kit is expensive, we did not investigate the effect of pH on IL-8 concentrations.

Discussion

This study demonstrates that IL-8 and TNF are present at high concentrations in gastric juice and biopsy homogenate supernatant fluid of patients infected with *H pylori*. Interleukin-8 concentrations were related to the severity of gastritis. Our findings suggest that increased local production of IL-8 and TNF may play an important role in the induction of the gastric mucosal damage associated with *H pylori* infection. Although IL-8 and TNF concentrations were elevated in the gastric juice and biopsy homogenate supernatant fluid of *H pylori* positive patients, concentrations of circulating IL-8 and TNF were unchanged. This dichotomy between local and systemic IL-8 and TNF responses indicates that the inflammatory response to *H pylori* infection mainly occurs at the local level in gastric tissue and that local production of these cytokines may occur.

As reported previously,[15] an inflammatory cell infiltrate, composed mainly of neutrophils, is present in *H pylori* associated gastroduodenal disease. The continued accumulation of neutrophils at the site of inflammation could be an important pathogenic mechanism.[17] The close histological association between *H pylori* and the inflammatory infiltrate is indicative of recruitment by inflammatory mediators. Our study suggests that increased local IL-8 production may be responsible for attracting neutrophils to the site of inflammation (*H pylori* also secretes a chemotactic factor for phagocytes[18 19]). Interleukin-8 is a potent and specific neutrophil chemotactic factor[5] and may play an important role in the pathogenesis of the inflammatory response associated with *H pylori* infection of the stomach and duodenum. Once the inflammatory cells are present, release of reactive oxygen radicals or proteolytic enzymes from stimulated neutrophils could induce tissue damage.[20]

Some studies have shown that TNF is an important mediator of inflammation and is closely related to the genesis of gastroduodenal inflammatory disease,[3 4] an observation further supported by this study. Tumour necrosis factor can stimulate production of IL-8 in vivo or in vitro.[5 21 22] The observation, therefore, that increased local production of IL-8 was positively correlated with TNF activity in patients with *H pylori* infection is not unexpected. Although the cellular source of IL-8 in gastric juice and biopsy homogenate supernatant fluid is uncertain, we postulate that gastric epithelial cells, infiltrating inflammatory cells, including macrophages, neutrophils, and lymphocytes, have the potential to produce large amounts of IL-8.[5 22-24]

In conclusion, our observations implicate inflammatory cytokines in the pathogenesis of *H pylori* associated gastroduodenal diseases. The emerging understanding of the potential role of IL-8 and TNF in inflammation may provide a new basis for the design of anti-inflammatory agents.

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