Interleukin 10 in *Helicobacter pylori* associated gastritis: immunohistochemical localisation and in vitro effects on cytokine secretion

K Bodger, K Bromelow, J I Wyatt, R V Heatley

Abstract

Background/Aims—Interleukin 10 (IL-10) is a counterinflammatory peptide implicated in the downregulation of human intestinal immune responses. Enhanced secretion of IL-10 has been documented in gastric biopsy organ culture in *Helicobacter pylori* infection. This study aimed to define the cellular origins of IL-10 in *H pylori* associated gastritis, and to determine the effects of endogenous IL-10 on proinflammatory cytokine secretion in vitro.

Methods—Endoscopic biopsies were obtained from the gastric antrum at endoscopy from patients with dyspepsia. Two pairs of antral biopsies were cultured in vitro for 24 hours, one pair in the presence of neutralising anti-IL-10 monoclonal antibody, the other pair as controls. The cytokine content of culture supernatants (tumour necrosis factor α (TNF-α), IL-6, and IL-8) was determined by enzyme linked immunosorbent assay and corrected for biopsy weight. *Helicobacter pylori* status was established by histology and biopsy urease test, and histopathology graded by the Sydney system. In a subgroup of patients, western blotting was used to establish CagA serological status. Immunohistochemistry for IL-10 was performed on formalin fixed tissues using a combination of microwave antigen retrieval and the indirect avidin–biotin technique. Immunoreactivity was scored semiquantitatively.

Results—In vitro culture was performed in 41 patients: 31 with *H pylori* positive chronic gastritis and 10 *H pylori* negative. In vitro secretion of TNF-α, IL-6, and IL-8 for "control" biopsies was significantly higher in *H pylori* positive versus negative samples, with values of TNF-α and IL-6 correlating with the degree of active and chronic inflammation and being higher in CagA seropositive cases. No evidence for enhanced cytokine secretion was seen in biopsies cocultured in the presence of anti-IL-10 monoclonal antibody. Immunohistochemistry was performed in 29 patients, of whom 13 were *H pylori* positive. IL-10 immunoreactivity was observed in the surface epithelium in all *H pylori* positive cases and in 13 of 16 negative cases, especially in areas of surface epithelial degeneration. Lamina propria mononuclear cells (LPMNCs) were positively stained in all *H pylori* positive cases and in 12 of 16 negative cases, with a significantly greater proportion of positive LPMNCs in the positive group.

Conclusions—This study localised IL-10 protein to the gastric epithelium and LPMNCs. In vitro proinflammatory cytokine secretion was increased in *H pylori* infection (especially CagA positive infection), but blocking endogenous IL-10 secretion did not significantly increase cytokine secretion. IL-10 is implicated in *H pylori* infection and might "damp down" local inflammation. The role of gastric IL-10 secretion in determining the clinicopathological outcome of infection merits further study.

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Division of Medicine, Level 7, Clinical Sciences Department, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK
K Bodger
K Bromelow
R V Heatley

Pathology Department, St James's University Hospital
J I Wyatt

Correspondence to: Dr Bodger, Department of Medicine, University Clinical Departments, Duncan Building, Daulby Street, Liverpool L69, UK
kbodger@compuserve.com

Despite the apparent activation of host cellular and humoral immune responses in *Helicobacter pylori* infection, colonisation of the gastric mucosa is generally persistent and results in chronic inflammation.[1] The severity of *H pylori* associated chronic gastritis and the clinical outcome are highly variable between individuals, with only a minority developing serious gastroduodenal disease. The factors that contribute to failure of the immune response to clear the organism and to the varied clinicopathological picture are poorly understood. The outcome of infection is likely to reflect a complex interaction between constitutional host factors, variation in virulence between infecting *H pylori* strains (for example, strains possessing the cagA pathogenicity island of genes),[2] and environmental cofactors.[1]

Cytokines are responsible for orchestrating the immune response, and we and others have reported the increased secretion of proinflammatory peptides (such as tumour necrosis factor α (TNF-α), interleukin 1 (IL-1) α/β, IL-6, and IL-8) in *H pylori* infection.[3–9] Less attention has been focused on the natural antagonists of these stimulatory cytokines. IL-10 is a potent anti-inflammatory cytokine with a variety of biological effects, including inhibition of many of the effector functions of phagocytes, such as the synthesis of proinflammatory cytokines (for example, TNF-α, IL-6, and IL-8).[10] The chronic enterocolitis developing in IL-10 (−/−) knockout mice suggests a role for this peptide in maintaining the normal non-inflamed state of the intestinal mucosa despite

continuous antigen exposure.[11] We postulated that IL-10 might contribute towards the failure of the immune response to clear *H pylori* infection, and in a previous study we found increased secretion of IL-10 in biopsy organ culture in *H pylori* infection, with secretion of the cytokine correlating with the degree of chronic inflammation.[3] Yamaoka *et al* have reported increased expression of IL-10 mRNA in biopsies from infected patients.[7 9] A role for IL-10 in "damping down" the immune response to helicobacter infection is supported by the recent observation of the rapid development of a severe hyperplastic gastritis in *Helicobacter felis* infected IL-10 (−/−) knockout mice.[12] The cellular sources of IL-10 and its effects on cytokine secretion in chronic *H pylori* infection are unknown.

In our present study, we aimed to: (1) define further the effects of IL-10 on proinflammatory cytokine secretion in *H pylori* infection; (2) correlate cytokine responses with histopathological features of gastritis and with CagA serological status; and (3) determine the cellular sources of IL-10 in the gastric antrum by means of immunohistochemistry.

Methods

PATIENTS AND BIOPSY SAMPLES FOR IN VITRO CULTURE

Consecutive patients with dyspepsia attending for diagnostic endoscopy were recruited, after obtaining informed consent. Patients who had received (within the last month) antibiotics, proton pump inhibitors, bismuth containing compounds, non-steroidal anti-inflammatory drugs, corticosteroids, or immunosuppressive drugs were excluded, as were patients with a known history of malignancy or immunosuppression. Our study was approved by the Leeds healthcare clinical research (ethics) committee.

During upper gastrointestinal endoscopy, multiple biopsies were taken from adjacent sites of the gastric antrum (within 3 cm of the pylorus), avoiding areas of macroscopic erosion or ulceration. Routine targeted biopsies were taken for diagnostic purposes using a standard procedure. Standard "pinch" biopsy forceps were used. Six antral biopsies were taken from each patient. Four biopsies were subject to in vitro culture (in pairs), one biopsy was fixed in 10% buffered formalin for histopathological processing, and one biopsy was used for biopsy urease testing (CLO test; Delta West Ltd, Perth, Western Australia). In addition, a 10 ml sample of venous blood was taken and serum was separated by centrifugation and stored at −20°C until subsequent western blot assay in a subgroup of patients.

HISTOPATHOLOGY

Serial 5 μm sections of formalin fixed, paraffin wax embedded sections were stained by both haematoxylin and eosin and modified Giemsa according to histopathological standards for light microscopy. Histology of routinely stained sections was subsequently reported by an experienced histopathologist (JIW) using the updated Sydney system,[13] blinded for the results of the cytokine estimations. A grade from zero (absent) to 3 (severe) was assigned for five histological parameters; namely, inflammation (chronic inflammatory cells), activity (neutrophils), glandular atrophy, intestinal metaplasia, and *H pylori* density.

DETERMINATION OF *H PYLORI* STATUS

Giemsa staining was used to identify *H pylori* in paraffin wax sections of formalin fixed antral biopsies, and the result of the biopsy urease test was read within 24 hours. The criteria for *H pylori* status were: negative, all tests negative; positive, at least one test positive.

IN VITRO ORGAN CULTURE

Culture medium comprised RPMI-1640 medium, supplemented with 10% fetal calf serum and antibiotics.[3] In the endoscopy suite, biopsies for in vitro culture were immediately placed in culture medium at 4°C and transported on ice to the laboratory. Biopsies were then placed in pairs in tissue culture wells containing 0.5 ml fresh culture medium as described previously.[3 14 15] One pair of biopsies from each patient was cultured in culture medium alone ("control" pair), whereas the remaining pair of biopsies was cultured in medium containing 1 μg/ml of mouse anti-human IL-10 neutralising monoclonal antibody (R&D Systems, Minneapolis, Minnesota, USA). This concentration was determined from the anticipated levels of endogenous IL-10 secretion (according to our previous work)[3] and reference to the manufacturer's data on the neutralisation capacity of the antibody, ensuring an excess of neutralising anti-IL-10 antibody.

Biopsies were then cultured for 24 hours at 37°C in a 5% CO_2 humidified incubator. At 24 hours, the culture medium was removed and the cells were centrifuged at 2000 ×*g* for 15 minutes at 4°C, snap frozen, and stored at −80°C until cytokine analysis. After culture, biopsies were dried to remove secreted mucus by gently turning on fresh, sterile filter papers and the wet weight determined as described previously.[3]

CYTOKINE ESTIMATIONS BY ENZYME LINKED IMMUNOASSAY

The cytokine content of culture supernatants was determined with the use of commercial microtitre plate enzyme linked immunosorbent assays (ELISAs), according to the manufacturers' instructions. All assays were based on a quantitative sandwich enzyme immunoassay technique. To allow multiple cytokine estimations, culture supernatants were prediluted in phosphate buffered saline (PBS; 1/4 to 1/64 depending on assay) before measuring the cytokine content, and values were adjusted for the dilution factor as appropriate. All assays were performed in duplicate. Cytokine secretion was normalised for biopsy wet weight and expressed as pg/mg/24 hours.

The ELISA assays all used a microtitre plate precoated with a monoclonal antibody directed against the cytokine to be assayed, to which samples and standards were added and plates were incubated. After washing, an enzyme

Table 1   Patient characteristics, histopathology (Sydney) scores, and cytokine secretion ("control" biopsies)

	Helicobacter pylori +ve	Helicobacter pylori −ve
Number	31	10
Mean age (range)	57.9 (32–84)	57.4 (23–87)
Male/female ratio	18/13	5/5
Antral inflammation		
Absent or mild (0, 1)	12/31	10/10
Moderate or severe (2, 3)	19/31	0/10
Antral activity		
Absent or mild (0, 1)	20/31	10/10
Moderate or severe (2, 3)	11/31	0/10
Cytokine secretion		
TNF-α	0.72* (0.27–1.20)	0.00 (0.00–0.18)
Interleukin 6	27.30* (14.30–67.30)	10.50 (5.65–12.72)
Interleukin 8	1122* (684–1864)	324 (269.5–533.7)

Values for cytokine secretion are median (interquartile range), expressed as pg/mg/24 hours.
*$p < 0.05$ for *H pylori* +ve *v* *H pylori* −ve (Mann Whitney U test).
TNF-α, tumour necrosis factor α.

linked polyclonal antibody specific for the cytokine was added and the plate was incubated and then washed again. A substrate solution was then added and after stopping the reaction the colour intensity was read by automated spectrophotometer. TNF-α was measured by means of a high sensitivity ELISA that uses an additional amplification step (HS "Quantikine"; R&D Systems), whereas IL-6 (Biotrak ELISA; Amersham Life Science, Amersham, Buckinghamshire, UK) and IL-8 ("Quantikine" ELISA; R&D Systems) were assayed by standard ELISA as described above. According to the manufacturers' data, the sensitivity of these assays were: TNF-α, < 0.11 pg/ml; IL-6, < 1 pg/ml; IL-8, < 10 pg/ml. No crossreactivity has been reported for other cytokines.

IMMUNOHISTOCHEMISTRY
Biopsy material comprised archival formalin fixed, paraffin wax embedded endoscopic antral biopsies from 29 cases of known *H pylori* status (by histology and urease testing), obtained from the pathology department, St James's University Hospital. Serial sections were subject to indirect immunohistochemistry using a combination of microwave antigen retrieval[16] and a standard indirect sequential avidin–biotin technique with 3,3'-diaminobenzidine tetrahydrochloride (DAB) development.[17]

Sections were dewaxed in xylene, rehydrated with graded alcohol, and washed in water. Endogenous peroxidase activity was blocked in 2% hydrogen peroxide in methanol and slides were washed. Pilot experiments established that specific staining was optimal using microwave antigen retrieval,[16 17] rather than untreated paraffin wax sections. Sections were subject to two incubation cycles of five minutes in plastic coplin jars containing citrate buffer (10 nmol/l citric acid, pH 6.0), using a laboratory microwave oven with an intrinsic temperature monitoring probe inserted into the buffer solution, and the temperature limit set to 80°C. Care was taken to ensure that biopsies were submerged in the buffer at all times. After cooling to room temperature for 20 minutes, sections were washed in PBS then incubated with rabbit serum buffer (1/20 in PBS) to block non-specific binding.

Sections were then incubated at room temperature in humidity chambers for two hours with optimal concentrations of primary antiserum (mouse antihuman IL-10 monoclonal antibody; R&D Systems) diluted in serum buffer. Pilot experiments using a range of dilutions (1/50 to 1/3200) of stock primary antiserum (0.5 mg/ml) established an optimal concentration for anti-IL-10 immunostaining (1/50). Normal serum buffer alone was used as a specificity control and all sections were negative when primary antiserum was omitted.

Slides were washed in running tap water, flooded with PBS, and drained. An aliquot of 50 μl of biotinylated secondary antibody (rabbit antimouse) was added to each section and slides were incubated for 30 minutes. Slides were again washed and 50 μl of peroxidase conjugated streptavidin was added to each slide and incubated for 30 minutes. The slides were washed again and developed for 10 minutes using fresh DAB solution. Sections were then counterstained with Mayer's haematoxylin for 30 seconds, washed in water, then dehydrated through alcohols. Finally, sections were cleared through xylene, mounted in DPX and subjected to light microscopy.

Immunostaining was graded semiquantitatively using a representative field viewed at high power. Separate scores were assigned for epithelium and lamina propria mononuclear cells (LPMNCs) as follows:
Epithelial staining: 0, absent; 1, weak; 2, moderate; 3, strong.
LPMNCs: (expressed as a proportion of positive cells) 0, absent; 1, scanty, isolated cells amounting to < 10% of total LPMNCs; 2, moderate numbers, > 10% but < 50% of total LPMNCs; 3, frequent, > 50% of LPMNCs.

DETERMINATION OF *H PYLORI* CagA STATUS
Sera from a subgroup of patients were subject to western blotting using a commercial kit for the identification of antibodies to the 120 kDa CagA protein (RIDA Blot Helicobacter IgG; Quadratech, Epsom, Surrey) according to the manufacturer's instructions. The kit contains nitrocellulose strips containing *H pylori* antigens that have previously been separated by weight using gel electrophoresis. Serum samples and positive and negative controls (supplied) are added to each strip in wells containing 2 ml of blotting buffer. Sealed wells are then incubated on a horizontal shaker and subsequently washed repeatedly. Enzyme conjugated antihuman IgG is then added, and after incubation the strips washed again. IgG from the sample/control serum that has bound to the protein bands on the strip is then visualised using a substrate solution (diaminobenzidine).

STATISTICAL ANALYSIS
Cytokine secretion is expressed as pg/mg/24 hours with median values and 25th to 75th interquartile ranges (IQR) given. Values for *H pylori* positive and negative patients (control biopsies only) were compared using the Mann-Whitney U test. Paired values (controls and biopsies cocultured with the anti-IL-10 monoclonal antibody) within the *H pylori* positive
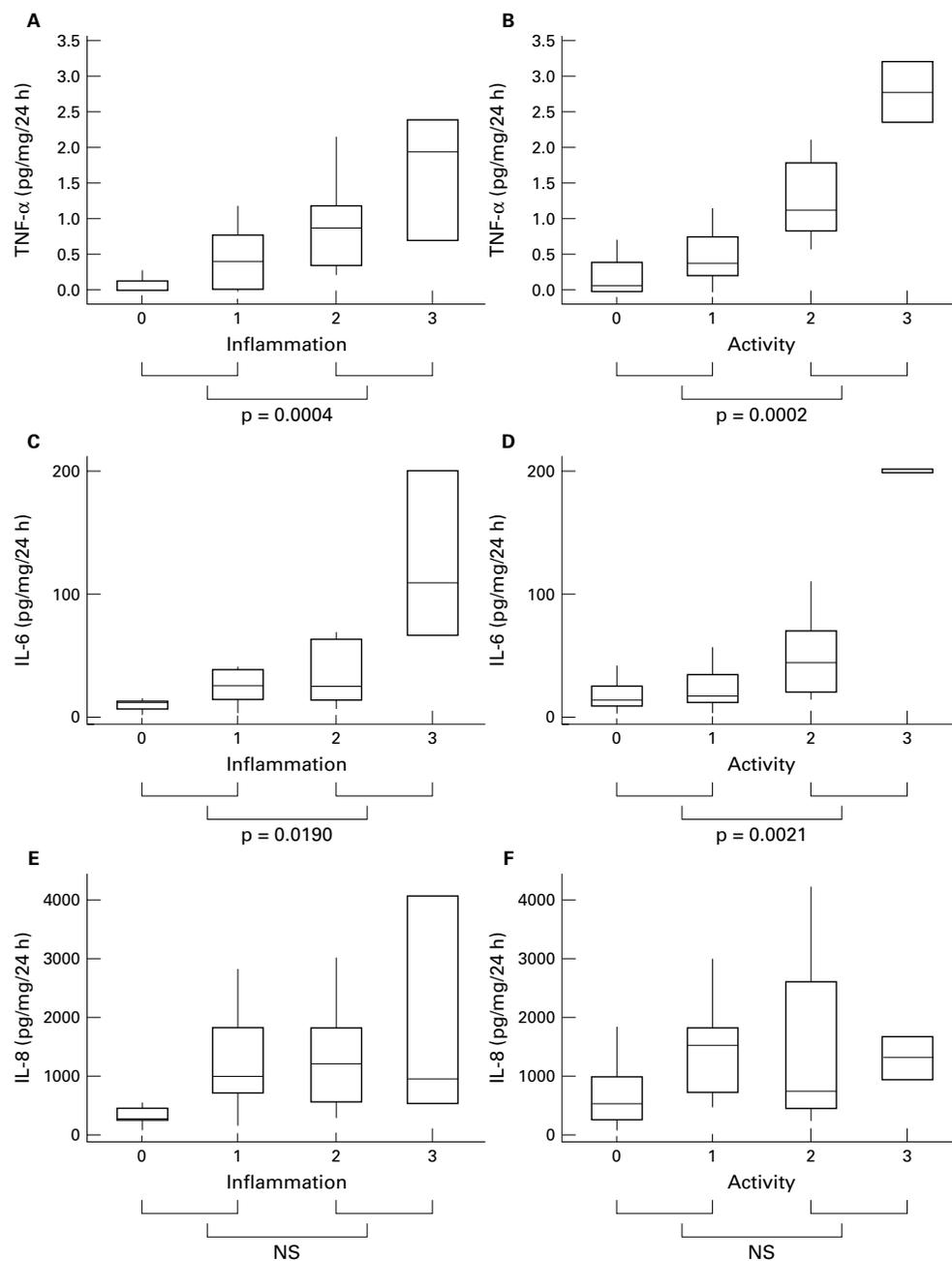
Figure 1   Concentration of tumour necrosis factor α (TNF-α) in 24 hour culture supernatants of "control" biopsies versus (A) antral inflammation (Sydney) score and (B) antral activity (Sydney) score. Concentration of interleukin 6 (IL-6) in 24 hour culture supernatants of "control" biopsies versus (C) antral inflammation (Sydney) score and (D) antral activity (Sydney) score. Concentration of interleukin 8 (IL-8) in 24 hour culture supernatants of "control" biopsies versus (E) antral inflammation (Sydney) score and (F) antral activity (Sydney) score.

group were compared using the Wilcoxon matched pairs test. Immunohistochemistry scores for *H pylori* positive and negative patients were compared using pooled score values by Fisher's exact test. All analyses used statistical software (Minitab v11.2, 1996; Minitab Ltd, Coventry, UK) and p values of ≤ 0.05 were regarded as significant.

Results

CYTOKINE SECRETION

Biopsies from 41 patients, of whom 31 were *H pylori* positive and 10 were negative on the basis of histology and biopsy urease testing of antral biopsies, were subject to in vitro culture.

Table 1 summarises the demographic and histological characteristics of the patients. The presence of the CagA protein was determined by western blotting in 18 *H pylori* positive patients, of whom 13 were CagA seropositive and five were CagA seronegative.

Table 1 and figs 1 and 2 summarise the in vitro cytokine secretion. Considering control biopsies alone, the proinflammatory cytokine content of culture supernatants (TNF-α, IL-6, and IL-8) was significantly greater in *H pylori* positive patients than in uninfected patients (table 1). The secretion of both TNF-α and IL-6 correlated both with the degree of chronic inflammation and with neutrophil activity, with
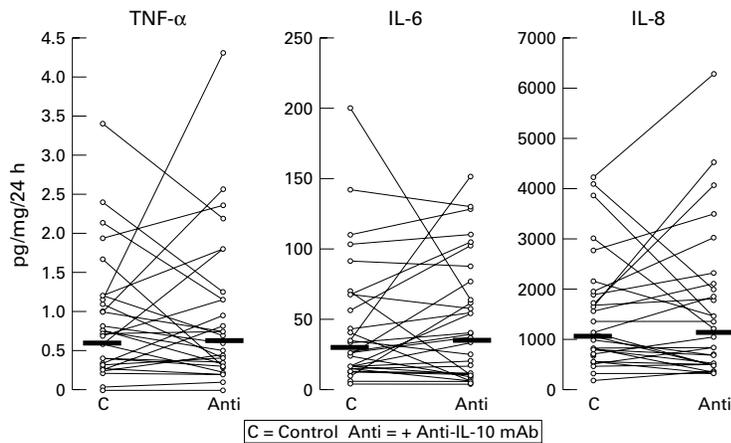
Figure 2   In vitro cytokine secretion for Helicobacter pylori positive patients: control biopsies versus biopsies cocultured in the presence of anti-IL-10 monoclonal antibody (mAb).

(0.295 to 1.435) and 0.04 (0 to 0.705) pg/mg/24 hours, respectively (p = 0.038, Mann Whitney U test). IL-6 secretion was also higher in the CagA seropositive subgroup, with a median (IQR) secretion of 23.8 (15.3 to 54.0) versus 13.20 (6.95 to 26.20) pg/mg/24 hours in the CagA seronegative group, although this did not reach significance (p = 0.11). There was no significant difference between the groups for IL-8 secretion, with median (IQR) values of 955 (717 to 1904) and 1650 (871 to 2252) pg/mg/24 hours, respectively (p = 0.55).

Figure 2 shows the effects of coculture with anti-IL-10 monoclonal antibody on cytokine secretion for the whole *H pylori* positive group (n = 31). Overall, there was no significant difference in proinflammatory cytokine secretion between control biopsies and those cocultured with neutralising anti-IL-10 monoclonal antibody. Stratifying the group by Sydney scores for the various histopathological parameters revealed no significant trends in the effects of coculture with anti-IL-10 monoclonal antibody. Indeed, secretion of TNF-α and IL-6 in the presence of anti-IL-10 monoclonal antibody showed the same association with histopathology scores as the control biopsies (fig 3), but with no evidence of heightened cytokine secretion. Cytokine secretion in
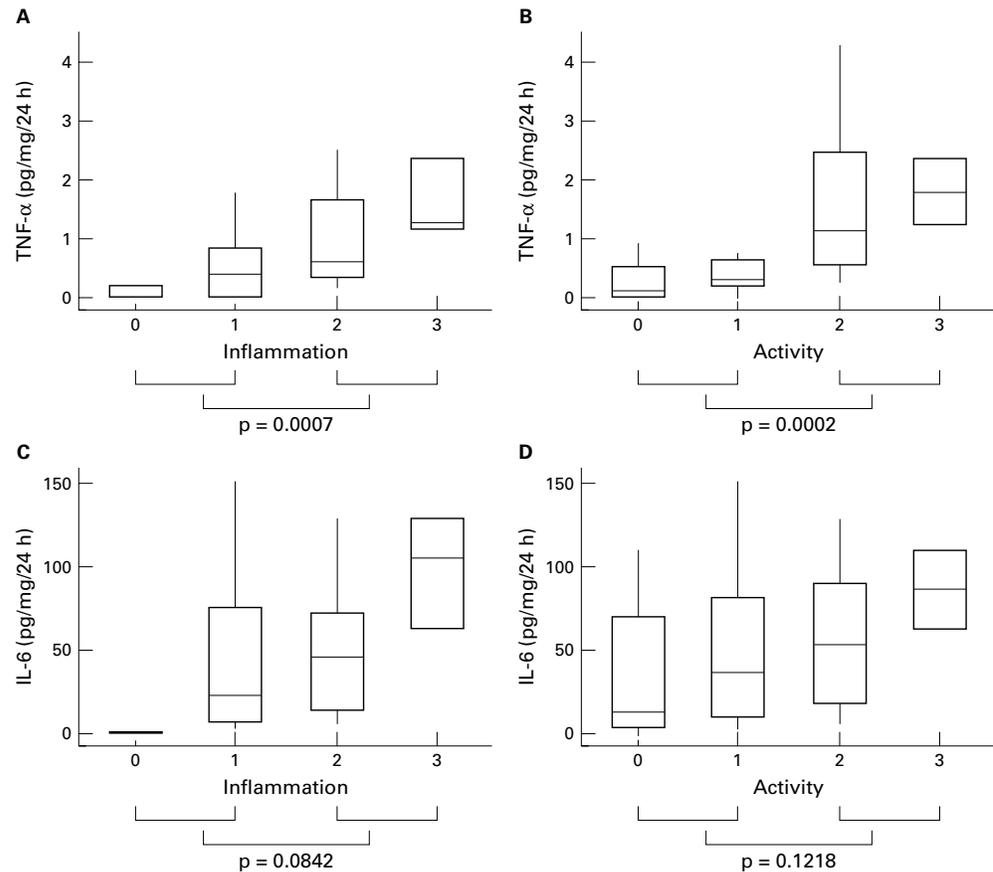
a significantly higher cytokine content in culture supernatants from patients having higher Sydney scores for these histopathological parameters (fig 1). There were no such trends for IL-8 secretion.

Within the subgroup of *H pylori* positive patients in whom the CagA serological status was determined, the TNF-α content of culture supernatants was higher in CagA seropositive cases (n = 13) than in seronegative cases (n = 5), with a median (IQR) secretion of 1.00

Figure 3   Concentration of tumour necrosis factor α (TNF-α) in 24 hour culture supernatants of biopsies cocultured in the presence of anti-IL-10 monoclonal antibody versus (A) antral inflammation (Sydney) score and (B) antral activity (Sydney) score. Concentration of interleukin 6 (IL-6) in 24 hour culture supernatants of biopsies cocultured in the presence of anti-IL-10 monoclonal antibody versus (C) antral inflammation (Sydney) score and (D) antral activity (Sydney) score.
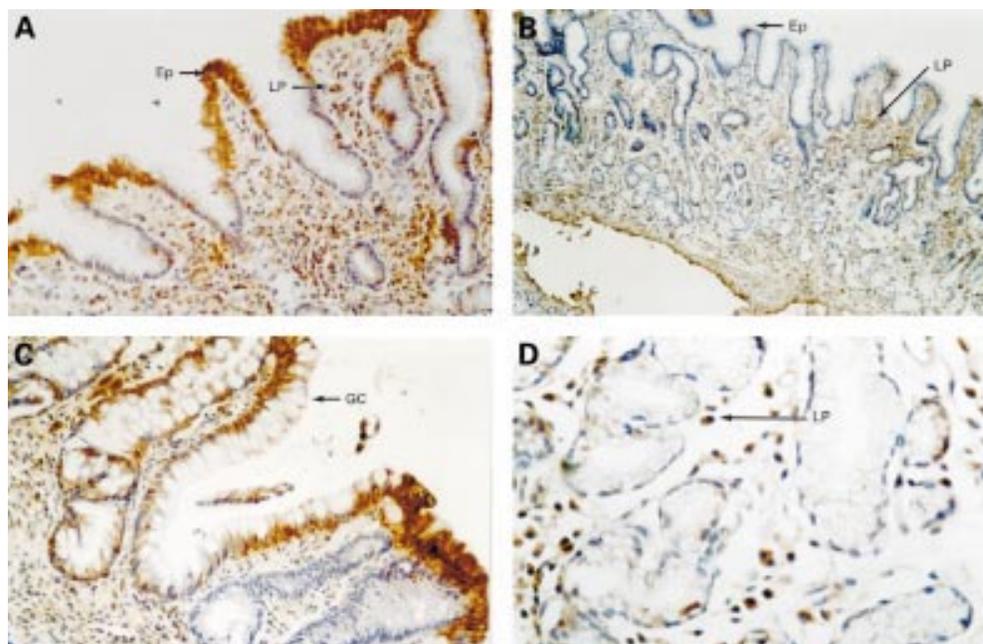
Figure 4   Immunohistochemical localisation of the interleukin 10 (IL-10) protein in formalin fixed gastric mucosal biopsies. (A) Helicobacter pylori positive patient: strong positivity in the surface epithelium (Ep) and frequent lamina propria mononuclear cells (LP). Original magnification, ×200. (B) An H pylori negative patient: weak focal positivity in surface epithelium (Ep) and scattered positive lamina propria (LP). Original magnification, ×100. (C) A patient with H pylori associated gastritis: strong cytoplasmic positivity of goblet cells (GC) in an area of intestinal metaplasia. Original magnification, ×200. (D) A patient with H pylori associated gastritis: high power view of lamina propria showing frequent positive lamina propria mononuclear cells (LP). Original magnification, ×400.

the presence of anti-IL-10 monoclonal antibody did not differ in relation to CagA status.

IMMUNOHISTOCHEMISTRY

Immunostaining for IL-10 was performed on formalin fixed, paraffin wax embedded sections of antral biopsies from 29 patients, of whom 13 were *H pylori* positive (chronic gastritis) and 16 were *H pylori* negative. Control sections (no primary antiserum) were uniformly negative in all cases.

All sections from *H pylori* positive patients and 13 of 16 *H pylori* negative patients studied showed some epithelial cell positivity (fig 4A and B). Diffuse cytoplasmic immunoreactivity of surface epithelial cells was apparent, focally extending down into foveoli. Staining was most intense in areas where surface epithelial degeneration was most severe. Foveoli were largely

Table 2   Expression of interleukin 10 (IL-10) protein by gastric epithelium and lamina propria mononuclear cells (LPMNCs) in Helicobacter pylori positive (chronic gastritis) and H pylori negative (normal mucosa) patients

	Helicobacter pylori +ve	*Helicobacter pylori −ve*
No. of cases studied	13	16
*Epithelium**		
Absent (0)	0	3
Weak (1)	1	3
Moderate (2)	7	8
Strong (3)	5	2
*LPMNCs***		
Absent (0)	0	4
<10% (1)	1	5
10–50% (2)	11	7
>50% (3)	1	0

*No significant difference between *H pylori* +ve *v H pylori* −ve.
**Proportion of LPMNCs stained for IL-10 protein: p < 0.05 for *H pylori* +ve *v H pylori* −ve (Fisher's exact test) using pooled values (scores 0–1 and 2–3).

spared and antral glands were generally negative, with occasional weak cytoplasmic positivity. In general, *H pylori* positive cases had more extensive epithelial staining than *H pylori* negative cases with normal mucosa, reflecting more widespread epithelial degeneration. Overall, the proportion of cases exhibiting absent/weak versus moderate/strong epithelial staining did not differ significantly with respect to *H pylori* status (table 2).

In sections containing areas of intestinal metaplasia (fig 4C), foci of complete intestinal metaplasia were either negative or showed weak positivity in the surface but not in the crypts. Incomplete intestinal metaplasia showed diffuse cytoplasmic positivity of non-goblet cell surface epithelium throughout, with less strong positivity in glands.

In all *H pylori* positive cases, some positive staining of lamina propria mononuclear cells (LPMNCs) was apparent (table 2; fig 4D), particularly in biopsies exhibiting more florid chronic inflammation. Positive cells were scattered diffusely in the lamina propria. Dense lymphoid aggregates were not stained. Positive LPMNCs were also seen in *H pylori* negative cases, although the proportion of positive cells was less than in *H pylori* positive cases. No immunostaining of neutrophils was seen.

Discussion

Our previous observation of IL-10 secretion from antral mucosa in *H pylori* infection led us to speculate that endogenous secretion of IL-10 might damp down the immune response to infection, limiting tissue damage but also potentially contributing towards the failure of the immune response to clear the organism.[3] In

our present study, we aimed to demonstrate the in vitro effects of endogenously released IL-10 on the production of proinflammatory peptides in *H pylori* infection and to determine possible sources of gastric IL-10 by means of immunohistochemistry.

Our present study confirms previous observations of enhanced proinflammatory cytokine release in *H pylori* associated chronic gastritis,[3–9] with significantly greater amounts of TNF-α, IL-6, and IL-8 in culture supernatants (control biopsies) from *H pylori* positive patients than from *H pylori* negative patients. The secretion of both TNF-α and IL-6 correlated with the degree of acute and chronic inflammation, reflecting enhanced cytokine production from more severely inflamed biopsies. We found enhanced production of TNF-α and IL-6 in a subgroup of *H pylori* infected patients whose sera contained antibodies to the CagA protein (compared with CagA seronegative cases). This was not significant in the case of IL-6 and secretion of IL-8 was not higher in CagA seropositive cases. Molecular analysis of gastric mRNA has suggested that infection with cagA+ strains is associated with increased transcription of IL-8, IL-1α, and IL-1β.[1 2 8 9]

Coculturing antral biopsies in the presence of neutralising anti-IL-10 antibody did not produce the expected increase in TNF-α, IL-6, and IL-8 release. There are several possible explanations for this. First, there are clear methodological limitations to our in vitro approach. Although the amount of anti-IL-10 antibody added to the culture medium would have been sufficient to block anticipated endogenous IL-10 release, penetration of the neutralising antibody into the biopsies might have been limited. Coculture of human intestinal biopsies has been used to study the effects of therapeutic agents on the release of inflammatory mediators in vitro,[14 15] but not to our knowledge in studying endogenous cytokine actions. Second, cytokine pathways are highly complex with individual peptides exhibiting diverse and often overlapping functions. The effects of blocking IL-10 bioactivity in organ culture might have been masked by secondary changes in the secretion of other cytokines, serving to sustain proinflammatory peptide secretion. In view of these limitations, an important role for IL-10 in reducing gastric immune responses to *H pylori* cannot be excluded. Recent observations of a rapidly progressive hyperplastic gastritis in IL-10 (−/−) knockout mice infected with a gastric helicobacter[12] argue in favour of our hypothesis that IL-10 does indeed play a key role in determining the severity of the local immune response in *H pylori* associated gastritis.

There has been considerable recent interest in the possible role of IL-10 in the gut. The chronic intestinal inflammation seen in the IL-10 (−/−) knockout mouse suggests that, in health, the peptide might reduce local inflammation stimulated by normal enteric antigen exposure.[11] Expression of IL-10 mRNA has been observed in normal colon tissues,[18] although the cellular source was not clear. Isolated colonic lamina propria T cells have been reported to release high amounts of IL-10 on activation by a variety of stimuli, suggesting one potential cellular source.[19] In a recent report, the in situ expression of IL-10 protein and mRNA was seen in both normal colon and in inflammatory bowel disease,[20] with expression localised to epithelial cells as well as LPMNCs.

Despite evidence of IL-10 expression/secretion by whole gastric biopsies,[3 7] there are no reports of in situ localisation of the cellular sources of production. Although originally described as a product of T helper type 2 (Th2) lymphocytes, IL-10 is produced by a variety of cell types, notably mononuclear phagocytes.[1 3] Present evidence suggests that a Th1 response predominates in *H pylori* infection,[21] such that the production of IL-10 by Th2 cells is unlikely to be a major source in gastritis.

In our present study, we localised IL-10 protein in formalin fixed, paraffin wax embedded gastric tissues using a combination of microwave antigen retrieval and standard peroxidase immunohistochemistry. Because of the absence of recognised control tissues for IL-10 immunostaining, we cannot be completely confident of the specificity of staining for IL-10. However, Autschbach *et al* have reported a close correlation between immunostaining of colonic tissue using the same primary antibody (MAB 217; R&D Systems) and the in situ expression of IL-10 mRNA, suggesting that immunostaining with this antibody provides accurate localisation of IL-10.[20]

We observed epithelial cell immunostaining for IL-10 protein in almost all sections, including both *H pylori* associated gastritis and normal mucosa. Although the strongest epithelial reaction was seen in *H pylori* positive cases in areas of epithelial degeneration, overall there was no significant difference in epithelial reactivity between inflamed biopsies (*H pylori* positive) and normal mucosa.

These findings are similar to previous observations of epithelial IL-10 protein and mRNA expression in both inflamed and non-inflamed colon.[20] Similarly, the proinflammatory chemokine, IL-8, can be demonstrated in gastric epithelial cells by means of immunohistochemistry in both normal and inflamed gastric tissues,[1 22] suggesting constitutive expression of immunoregulatory peptides in human gastric epithelium. Epithelial IL-10 secretion could reduce local immune responses against luminal antigens, contributing to a state of "controlled inflammation" in the normal intestine,[23] and influencing the clinicopathological outcome of mucosal infection.

Conclusion

IL-10 is a potent counterinflammatory peptide implicated in the downregulation of human intestinal immune responses. Our study localised IL-10 protein in situ to the gastric epithelium and LPMNCs in both normal mucosa and *H pylori* gastritis, a phenomenon not reported previously. Although blocking IL-10 bioactivity in organ culture did not produce enhanced proinflammatory peptide release from the antral mucosa, further studies of the

role of IL-10 in *H pylori* infection are required. In agreement with previous studies, we have shown that proinflammatory peptides such as TNF-α, IL-6, and IL-8 are secreted in *H pylori* gastritis, and our data demonstrate that the secretion of TNF-α and IL-6 correlate with increased severity of acute and chronic inflammation, and that concentrations of these molecules are increased in patients infected with cagA positive strains in particular.

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