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Up-Regulation of IL-17 Is Associated with Bioactive IL-8 Expression in Helicobacter pylori-Infected Human Gastric Mucosa

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Helicobacter pylori (Hp) secretion is associated with an increased number of acute and chronic inflammatory cells secreting cytokines that contribute to maintain and expand the local inflammation. Locally induced IL-8 is believed to play a major role in the Hp-associated acute inflammatory response. Factors/mechanisms that regulate IL-8 induction are, however, not fully understood. In the present study we investigated whether Hp infection is associated with an increased production of IL-17, a T cell-derived cytokine capable of modulating IL-8 gene expression. We showed that both IL-17 RNA transcripts and protein were expressed at a higher level in the whole gastric mucosal and lamina propria mononuclear cell samples from Hp-infected patients than in those from uninfected subjects. Hp eradication was associated with a marked down-regulation of IL-17 expression. The addition of a neutralizing anti-IL-17 Ab to the gastric lamina propria mononuclear cell cultures resulted in a significant inhibition of IL-8 secretion, indicating that IL-17 contributes to enhance IL-8 in the Hp-colonized gastric mucosa. Consistently, stimulation of MKN 28 cells, a gastric epithelial cell line, with IL-17 increased IL-8 secretion. Finally, conditioned medium from the IL-17-stimulated MKN 28 cell cultures promoted the in vitro polymorphonuclear leukocyte migration. This effect was inhibitable by a neutralizing IL-8 but not IL-17 Ab. Together, these data indicate that biologically active IL-17 production is increased during Hp infection, suggesting the possibility that this cytokine may play an important role in the inflammatory response to the Hp colonization. The Journal of Immunology, 2000, 165: 5332–5337.

Helicobacter pylori (Hp) is recognized as the most common cause of chronic active gastritis and as an important pathogenic factor in peptic ulcer disease. Hp-associated inflammatory reaction is characterized by a massive mucosal infiltration of polymorphonuclear leukocytes (PMN), T cells, macrophages, and plasma cells (1). These cellular changes are associated with enhanced production of cytokines, which are believed to contribute to maintaining the gastric inflammation and causing epithelial cell damage (1).

In recent years considerable evidence has been accumulated to indicate that IL-8, the major human PMN chemoattractant, plays a major role in the Hp-associated acute inflammatory response (2–4). Both IL-8 mRNA and protein levels strictly correlate with Hp density (5, 6). In addition, IL-8 expression has been associated with significantly more severe infiltration of PMN, and down-regulation of mucosal IL-8 synthesis, induced by Hp eradication, is paralleled by a resolution of the PMN infiltration (7, 8). During Hp infection, epithelial cells are the major producers of IL-8, even if there is evidence that macrophage-like cells synthesize IL-8 (3). Although several studies have documented the ability of Hp to directly stimulate IL-8 synthesis, there is evidence that T cell-derived cytokines may modulate epithelial cell IL-8 gene expression (9).

IL-17 is a recently described cytokine encoded by a 155-aa open reading frame that includes an N-terminal secretion signal sequence of 19–23 residues (10). IL-17 is produced by activated CD4+ T lymphocytes, mainly Th0 and Th1 cells (11), and exhibits pleiotropic biological activities on various cell types, including macrophages, fibroblasts, and endothelial and epithelial cells (12). IL-17 has been found to stimulate the synthesis of IL-1β, IL-6, TNF-α, PGE2, ICAM-1 and cyclo-oxygenase-2 (13, 14). Thus, IL-17 appears to provide a link between T cell activation and inflammatory responses. Consistently, an enhanced expression of IL-17 has been documented and implicated in the pathogenesis of immune-mediated diseases, such as rheumatoid arthritis, multiple sclerosis, and psoriasis (14). Moreover, IL-17 has the ability to stimulate IL-8 production in both epithelial cells and macrophages (13, 15), raising the possibility that this cytokine may play an important role in the recruitment of inflammatory cells during bacterial infections.

Knowing that activation of CD4+ Th1 cells is a key feature of Hp infection (16–18), we explored the hypothesis that the locally induced IL-17 may be involved in the local inflammatory response to gastric Hp colonization. In this work we report for the first time that biologically active IL-17 production is increased in the mucosa of Hp-infected patients. In addition, we show that IL-17 is capable of stimulating IL-8 release by gastric epithelial cells and favoring PMN chemotaxis through an IL-8-dependent mechanism.
These data suggest that IL-17 may play an important role in the maintenance of the mucosal inflammatory response during Hp infection.

Materials and Methods

Patients and sampling

Thirty-six patients (18 men and 18 women; age range, 19–71 years; median, 39 years) who underwent esophagogastroduodenoscopy for dyspeptic symptoms were studied. Endoscopic findings were as follows: normal mucosa or mild gastric erythema, 26 patients; esophagitis, five patients; erosive duodenitis, two patients; duodenal ulcer, two patients; and erosive gastritis, one patient. Twenty-nine patients had evidence of Hp infection. Nine Hp-infected patients were re-endoscoped 1 mo after cessation of 1-wk treatment with lansoprazole (60 mg twice daily), tinidazole (500 mg twice daily), and clarithromycin (500 mg twice daily). Six of these nine patients were Hp eradicated. Seven of the 36 patients were Hp negative. Mucosal samples, with no evidence of macroscopic and histological lesions, were taken from seven of these patients and considered as normal tissue. During endoscopy eight gastric biopsy specimens were taken: one from the antrum for urease quick test (Yamanouchi Pharma, Milan, Italy), four from the antrum and corpus for histological examination, and three from the antrum for cytokine measurement. All 36 patients were used as sources of tissue for Hp-positive and Hp-negative patients. Autologous PBMC were obtained from three Hp-infected and three uninfected patients. At this time a blood sample was drawn from each patient. No patient had previously undergone anti-Hp treatment or had received antibiotics within the previous 3 mo. Informed consent was obtained in all patients, and the protocol was approved by the local ethical committee.

Histological examination

Sections of biopsy specimens were embedded in paraffin and stained with hematoxylin and eosin to examine gastritis and with Giemsa to detect Hp. The degree and activity of gastritis and the density of Hp colonization, atrophy, and intestinal metaplasia were graded according to the Sydney system (19) on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes.

Determination of Hp status

Patients were classified as Hp infected when the urease quick test result was positive and/or the organism was identified in the Giemsa-stained sections in the antral and/or corpus gastric samples. A 13C urea breath test (Cortex, Milan, Italy) was also performed, and successful eradication of Hp was determined if all test results were negative.

Gastric lamina propria mononuclear cell (LPMC) and MKN 28 cell cultures

Gastric LPMC were isolated by the DT-T-EDTA-collagenase sequence as previously described in detail (20). The resulting cell preparations contained <5% contaminating epithelial cells. The isolated cells were counted, and checked for viability using 0.1% triypan blue (viability ranged from 90–94%). Gastric LPMC were resuspended in complete medium (RPMI 1640 supplemented with 10% FCS, 1% l-glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin; all obtained from Sigma, St. Louis, MO) at a concentration of 2 × 106 cells/ml and were cultured in the presence or the absence of a neutralizing anti-IL-17 Ab (at a final concentration of 1, 10, or 100 ng/ml) or a relevant control Ab (anti-IL-4 Ab, 1 µg/ml; both purchased from R&D Systems, Minneapolis, MN) for 18 h. PBMC were also isolated by density gradient centrifugation (Lymphoprep, Nycomed Pharma, Oslo, Norway) from 10-ml heparinized blood samples. To examine whether IL-17 enhances IL-8 secretion in gastric epithelial cells, MKN 28 cells (gift from Dr. Zarrilli, University of Naples, Naples, Italy) were resuspended in DMEM supplemented with 10% FBS, 2 mmol glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin (all obtained from Sigma) at a concentration of 3 × 106 cells/ml and were cultured until they reached confluence. After that, MKN 28 cells were stimulated with or without graded doses of recombinant human IL-17 (rHL-17; final concentrations, 1, 10, and 100 ng/ml; R&D Systems) for 6, 12, 18, and 24 h.

Gastric tissue homogenate preparation for RNA analysis

Gastric biopsy specimens were used for RNA and protein analysis of freshly obtained whole tissue. Mucosal samples were separately placed in sterile tubes containing 1–2 ml of cold guanidine thiocyanate buffer (for RNA extraction) or 0.5 ml of lysis buffer (for protein extraction). The latter contained 0.0625 mol/L Tris (pH 6.8), 2% SDS, 3% 2-ME, 10% glycerol, 100 mmol/L sodium fluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin, and 1 mmol/L PMSF (all from Sigma). Tissue samples were homogenized using a tissue homogenizer (model no. D-7801, Ystral, Dortmung, Germany).

RNA and cDNA preparations

Total RNA was extracted from mucosal samples according to the method of Chomczynski and Sacchi (21). The sample obtained was quantitated by absorbance at 260 nm. RNA integrity was assessed by electrophoresis on a 1.5% agarose gel. cDNA was synthesized from 1 µg of total RNA using 0.2 U of murine leukemia virus reverse transcriptase (Promega, Madison, WI) and 50 µM random hexamers, combined with 50 pmol dNTP (Roche), and 2 U of RNase inhibitor (Promega) in a total volume of 20 µl. The reaction was performed at 37°C for 60 min.

RT-PCR

Before examining transcripts for IL-17 and IL-17R, the sample cDNA content was normalized with a β-actin signal. For this purpose, varying amounts of cDNA were incubated in a PCR for 19, 20, 21, 22, and 23 cycles with β-actin-specific primers. IL-17 and IL-17R primers were assayed in all samples by incubating an equivalent amount of cDNA for 35 cycles. PCR were performed in a total volume of 50 µl in the presence of 1 U of Taq DNA polymerase (Roche), 200 mmol of dNTPs (Roche), and 25 pmol/L 5’ and 3’ primers. Reactions were incubated in a Robocycler thermal cycler (Stratagene, La Jolla, CA; denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C).

PCR primers

IL-17 primers (Genosys, Cambridge, U.K.) were as follows: β-actin, 5’-GGAGGCCCAGAGCAAGAGA-3’ and 3’-GGTGACATTTAAGGAGAAGCTTGTTG-5’; IL-17-β, 5’-GGTACCTACATGAACTC-3’ and 3’-CAATCTTGGCAATGTTTAC-5’; and IL-17R, 5’-GGCTACCTGGGTGAAGGATAC-3’ and 3’-GGGATGTCCGATGTAAGAC-5’.

To exclude the amplification of genomic DNA contaminating the samples, experiments were performed using RNA as substrate for PCR assay. Ten microliters of PCR product was combined with 1 µl of loading buffer and electrophoresed on a 1.5% agarose gel (in Tris-EDTA buffer). A 123-bp ladder was used to assess sample size. The specificity of the PCR products was confirmed by specific restriction enzymes.

Southern blotting

To assess IL-17 semiquantitatively, Southern blotting analysis was performed as previously indicated (22). In preliminary experiments we established the optimal number of RT-PCR cycles for obtaining a product within the linear portion of the curve. An equivalent amount of cDNA was therefore incubated for 18 or 22 cycles with β-actin- or IL-17-specific primers, respectively. PCR were performed in a total volume of 50 µl. The PCR products were detected by Southern blot hybridization using specific cDNA probes. The cDNA probes were DNA fragments encoding the full-length PCR product. RT-PCR products were used as probes only after each product was cloned, and its sequence verified. The level of RNA transcripts was measured by laser densitometry (NIH Image software) and expressed as arbitrary units.

Protein extraction and Western blot analysis

Total proteins were extracted from both freshly obtained mucosal samples and LPMC by using the above mentioned lysis buffer. After cell lysis, the supernatant was collected, run at 4000 × g for 40 min (4°C), and stored at −80°C until assay. Total proteins were separated on a 15% SDS-PAGE gel and electrophoretically transferred onto an Immobilon-P membrane (Amersham, Aylesbury, U.K.). IL-17 was detected using an anti-IL-17 (1/5000 dilution), capable of recognizing the carboxyl terminus of IL-17 (Santa Cruz Biotechnology, Santa Cruz, CA), followed by an HRP-conjugated goat anti-mouse IgG mAb (Santa Cruz Biotechnology; final dilution, 1/5000). Ab reactions were detected with a chemiluminescence detection kit (Amersham). To confirm the equal loading of proteins, Ponceau S staining was performed.

IL-8 ELISA

IL-8 was measured in LPMC and MKN 28 supernatants using a sensitive ELISA (R&D Systems). The minimum detectable IL-8 concentration was 5 pg/ml.

Assessment of IL-17 effects on PMN chemotaxis

Dextran-separated PMN, isolated from healthy donors, were resuspended in DMEM at a final concentration of 3 × 106 cells/ml. The chemotaxis assay was performed in a 48-well microchemotaxis chamber (Corning-Costar, Cambridge, MA) as previously described (23). Briefly, the bottom...
wells of the chamber were filled with 25 μl of fluid containing either rhIL-8 (at a final concentration of 1 ng/ml), the control solution (DMEM with 1% BSA), the conditioned medium (IL-17-stimulated MKN 28 cell culture supernatant), or IL-17 (100 ng/ml). A filter with pore size of 3 mm was placed on the bottom wells, and 50 μl of neutrophil suspension were pipetted into upper wells as triplicate samples. The chamber was incubated in humidified air with 5% CO2 at 37°C for 30 min. PMN that completely petted into upper wells as triplicate samples. The chamber was incubated and Methods

blot analysis of RT-PCR products was performed as indicated in

Patients with a median antral score of 4 exhibited levels of IL-17

specific Ab that is capable of recognizing the carboxyl terminus of

RNA, total proteins extracted from freshly obtained mucosal tissue

were present in both whole mucosal gastric and LPMC samples (Fig. 2).

To determine whether human gastric T cells translate IL-17 RNA, total proteins extracted from freshly obtained mucosal tissue and LPMC samples were analyzed by Western blotting using a specific Ab that is capable of recognizing the carboxyl terminus of IL-17. In all Hp-infected patients and normal controls, the anti-IL-17 Ab detected a protein with a molecular size of ~20 kDa (Fig. 3). This polypeptide comigrated with rhIL-17 upon SDS-PAGE (Fig. 3). The amount of IL-17 detected in the mucosa of patients with Hp infection was greater than that found in normal mucosal samples (Fig. 3). This was evident in both whole mucosal tissue homogenates and LPMC (Fig. 3). As Ponceau S staining confirmed that similar amounts of proteins were loaded into each lane, these results indicate that up-regulation of IL-17 occurs in the Hp-colonized gastric mucosa. A more pronounced expression of IL-17 was seen in mucosal samples with a higher gastritis score. Patients with a median antral score of 4 exhibited levels of IL-17 (median, 232 densitometry arbitrary units; range, 221–290) lower than those measured in patients with a median score of 7 (median, 421; range, 303–592; p < 0.001, by Mann-Whitney U test). In addition, in Hp-infected patients with the same gastritis score, IL-17 levels positively correlated with the density of Hp (r = 0.9; p < 0.001, by Pearson’s correlation test). Taken together, these data suggest that IL-17 production is in part dependent on the grade of T cell infiltration and that Hp infection may have a stimulatory effect on IL-17 induction. To further support this concept, IL-17 was analyzed in mucosal samples taken from six patients before and after a successful eradication treatment. As shown in Fig. 4, Hp eradication was associated with a marked down-regulation in IL-17. This was associated with a significant decrease in gastritis score and number of infiltrating PMN (data not shown).

IL-17 produced in the gastric mucosa of Hp-infected patients is biologically active

The demonstration that gastric LPMC contain transcripts for IL-17R suggests that these cells are potential targets of IL-17 in vivo. Evidence has accumulated to indicate that both epithelial cells and macrophage-like cells infiltrating the Hp-colonized mucosa produce IL-8 (3). In addition, it has been reported that IL-17 is capable of inducing IL-8 in both epithelial cells and macrophages in other systems (14, 15). We therefore examined the ability of the locally produced IL-17 to modulate IL-8 production in gastric LPMC. For this purpose, LPMC were isolated from the stomach of patients with Hp infection and incubated with graded doses of a neutralizing anti-IL-17 Ab. After 18 h of culture, the amount of IL-8 released in the LPMC culture supernatants was measured by ELISA. As shown in Fig. 5 the addition of an anti-IL-17 Ab to the LPMC cultures down-regulated IL-8 secretion. The amounts of IL-8 released by LPMC cultured in the presence of 1, 10, and 100 ng/ml anti-IL-17 Ab were 21, 50, and 59% lower than those measured in unstimulated LPMC culture supernatants (p < 0.01, by

Results

IL-17 expression is enhanced in the gastric mucosa of patients with Hp infection

Transcripts for IL-17 were detected in the gastric tissue homogenates from both Hp-infected patients and uninfected subjects. When IL-17 RNA transcripts were analyzed by semiquantitative RT-PCR, an increased accumulation was seen in whole mucosal tissue from patients with Hp infection compared with uninfected subjects (Fig. 1). Consistently, a more pronounced accumulation of IL-17 RNA transcripts was detected in LPMC isolated from the gastric mucosa of patients with Hp compared with normal controls (Fig. 1). IL-17 was barely detectable in PBMC, with no difference between Hp patients and controls. Transcripts for IL-17R were present in both whole mucosal gastric and LPMC samples (Fig. 2).

Differences between groups were compared with Student’s t test and Mann-Whitney U test. IL-17 blots were scanned using laser densitometry analysis (NIH Image software). The resulting arbitrary units were correlated with gastritis and Hp density scores by means of Pearson’s correlation test. p < 0.05 was considered statistically significant.

Statistical calculations

FIGURE 1. IL-17 RNA transcripts are increased in the gastric mucosa of Hp-infected patients. RNA was extracted from whole mucosal gastric (lanes 1, 3, 5, and 7) and LPMC (lanes 2, 4, 6, and 8) samples from two Hp-negative (lanes 1–4) and two infected patients (lanes 5–8). cDNA samples (1.5 μl for β-actin and 3 μl for IL-17) were incubated with specific primers for β-actin and IL-17 for 18 and 22 cycles, respectively. Southern blot analysis of RT-PCR products was performed as indicated in Materials and Methods. One of three representative experiments is shown.

FIGURE 2. Agarose gel showing RT-PCR product for IL-17R (331 bp) and β-actin (485 bp) in tissues from both uninfected (lanes 2 and 3) and Hp-infected (lanes 4 and 5) patients. In these tissues, IL-17R RNA transcripts were examined and detected in both whole gastric mucosal (lanes 2 and 4) and LPMC (lanes 3 and 5) samples. Transcripts for IL-17R were also present in MKN 28 cell lines (lane 6). Lane 1 indicates a 123-bp ladder, whereas lane 7 shows a negative control tube (RT-PCR product obtained by using no cDNA sample in the PCR). Total RNA, extracted as indicated in Materials and Methods, was used for cDNA preparation. cDNA samples were amplified using specific primers for IL-17R (after 35 cycles) and β-actin (after 23 cycles). One representative experiment of six independent experiments is shown.

FIGURE 3. Western blot analysis of IL-17 in freshly obtained mucosal tissue (lanes 1, 3, 5, and 7) and LPMC (lanes 2, 4, 6, and 8) from two Hp-uninfected (lanes 1–4) and two Hp-infected (lanes 5–8) patients. Anti-IL-17 Ab detected a protein corresponding to the size of rhIL-17 (lane 9). One representative experiment of six independent experiments is shown.
Student’s paired, two-way t test; n = 6; 1, 10, and 100 ng/ml vs basal secretion; Fig. 5). The effect of anti-IL-17 appeared to be specific. No inhibition of IL-8 production was seen when LPMC were cultured in the presence of control Ab. In addition, LPMC viability was not affected by anti-IL-17 treatment.

**IL-17 enhances IL-8 production in MKN 28 cells and promotes the in vitro chemotaxis of PMN**

To further substantiate the role of IL-17 in the induction of IL-8 in gastric mucosa, we tested the effect of rhIL-17 on secretion of IL-8 by MKN 28 cells, a gastric epithelial cell line. We first showed that MKN 28 cells express transcripts for IL-17R. This is consistent with the demonstration that IL-17R is expressed in several cell types, and epithelial cells are potential targets of IL-17 (12–15). Indeed, stimulation of MKN 28 cells with rhIL-17 was followed by an increase in IL-8 secretion (Fig. 6). This effect was dose dependent. The addition of 1, 10, and 100 ng/ml IL-17 increased IL-8 secretion of 44, 138, and 280% (p < 0.01, n = 6; 10 and 100 ng/ml vs basal secretion; p = NS, n = 6, 1 ng/ml vs basal secretion; by Student’s paired, two-way t test; Fig. 6). The enhancing effect of IL-17 on IL-8 was neutralized by coincubation with anti-IL-17, but not control, Ab.

Recent research has raised the possibility that IL-17 can recruit PMN into the mucosa through the induction of IL-8, providing a link between the activation of T lymphocytes and the recruitment of acute inflammatory cells (15). To investigate whether this mechanism is also operating at the gastric level, we tested the in vitro migration of human PMN in response to the stimulation with culture supernatants

**FIGURE 4.** Western blot analysis of IL-17 in freshly obtained mucosal tissue from three patients before (lanes 1–3) and after (lanes 4–6) a successful eradication treatment. One representative experiment of two independent experiments is shown.

**FIGURE 5.** Neutralization of endogenous IL-17 results in a down-regulation of IL-8 secretion in gastric LPMC cultures. LPMC were cultured, as indicated in Materials and Methods, with graded doses of a neutralizing anti-IL-17 Ab for 18 h. The IL-8 level in the culture supernatants was measured by ELISA (*, basal secretion vs 1, 10, and 100 ng/ml, p < 0.01). The effect of a control Ab (anti-IL-4, 1 μg/ml) is also shown (§, control Ab vs basal secretion, p = NS). Data are the mean ± SD of six experiments. Statistical significance was assessed by Student’s paired, two-way t test.

**FIGURE 6.** IL-17 stimulates IL-8 synthesis in MKN 28 cells. MKN 28 cells were cultured with graded doses of rhIL-17 for 18 h, and the level of IL-8 in the culture supernatants was measured by ELISA. Addition of IL-17 to the MKN 28 cell cultures resulted in a dose-dependent increase in the secretion of IL-8 compared with that measured in unstimulated cell culture supernatants (*, 10 and 100 ng/ml vs basal secretion, p < 0.01; §, 1 ng/ml vs basal secretion, p = NS). The effects of coincubation with a neutralizing anti-IL-17 Ab and with a control Ab (anti-IL-4, 1 μg/ml) are also shown (▲, p < 0.01; *, p = NS vs basal secretion, respectively). Data are the mean ± SD of six experiments. Statistical significance was assessed by Student’s paired, two-way t test.

**FIGURE 7.** Human PMN migration in chemotaxis multiwell chamber caused by 30-min stimulation with culture supernatant of MKN 28 cells treated with IL-17 (100 ng/ml) for 18 h (conditioned medium, CM) or rhIL-8 (1 ng/ml). CM medium caused a significant increase in PMN migration compared with the vehicle (DMEM; *; p < 0.01). When CM was coincubated with a neutralizing anti-IL-8 Ab, the number of PMN that migrated was not different from that observed in the presence of vehicle alone (§, p = NS). In contrast, no inhibitory effect on CM-induced PMN migration was attributable to an anti-IL-17 Ab (#, CM plus anti-IL-17 vs vehicle, p < 0.01). Human rIL-8, but not rh-IL-17 induced a significant increase in PMN migration (▲, p < 0.01; *, p = NS vs vehicle, respectively). Data are the mean ± SD of four experiments and refer to the number of PMN per light microscope HPF. Statistical significance was assessed by Student’s paired, two-way t test.
of IL-17-treated MKN 28 cells (conditioned medium). As shown in Fig. 7, the incubation with conditioned medium significantly enhanced PMN migration. To determine whether this effect was mediated by IL-8 induction, PMN were incubated with conditioned medium in the presence or the absence of a neutralizing anti-IL-8 or IL-17 Ab. As shown in Fig. 7, the anti-IL-8, but not anti-IL-17, Ab inhibited the in vitro chemotaxis of conditioned medium-induced PMN. Interestingly, no increase in PMN migration was observed when PMN were treated with rhIL-17 (Fig. 7).

Discussion

The present study was designed to explore the expression of IL-17 during Hp infection. We show that biologically active IL-17 production is enhanced in the Hp-colonized gastric mucosa.

IL-17 is a recently described cytokine that is capable of modulating the expression of various genes in fibroblasts, macrophages, and epithelial and endothelial cells and is implicated in the pathogenesis of human inflammatory diseases (10, 14, 24–26). Of the large number of cell types analyzed for IL-17 expression, it is the T lymphocyte that appears to be the only producer of IL-17 (10, 11). In particular, IL-17 is produced by CD45RO+T cells, but not CD45RA+T cells (27). We report that LPMC isolated from the human gastric mucosa express IL-17 at both RNA and protein levels. This appears to be consistent with the demonstration that the vast majority of lymphocytes infiltrating the gastrointestinal mucosa bear the phenotype of Ag-experienced memory T cells (20, 23–30).

When RNA transcripts for IL-17 were analyzed by a semiquantitative RT-PCR, an increased accumulation was found in both mucosal tissue and LPMC samples from Hp-infected patients compared with that in uninfected subjects. Similarly, the amount of IL-17 protein, as determined by Western blotting, was increased in the mucosa of Hp-infected patients. In addition, Hp eradication resulted in a down-regulation of IL-17 production. As no difference in terms of IL-17 expression was seen in blood lymphocytes isolated from patients with Hp and controls, it is highly likely that the mucosal microenvironment, determined by the bacteria infection, influences IL-17 expression. Among T cells, the activated Th1/Th0 cell subset is the major source of IL-17 (11). Consistently, IL-17 has been found to be increased in Th1-mediated diseases (14, 24, 25).

The greater expression of IL-17 seen in Hp-positive biopsies compared with that found in Hp-negative samples may depend on the more pronounced infiltration of memory T cells in the Hp-colonized mucosa. This is also consistent with our finding of a positive correlation between IL-17 levels and gastritis score in the Hp-positive group. Analysis of IL-17 in other forms of gastritis by comparison with Hp-associated gastritis would be useful to establish whether up-regulation of IL-17 specifically occurs during Hp infection or simply reflects a general phenomenon of all forms of gastritis. Unfortunately, in the geographical area where we work, the high prevalence of Hp infection in the population undergoing esophagogastroduodenoscopy makes it extremely difficult to sample biopsies from Hp-negative gastritis. The fact that in the Hp-positive patients matched for the level of gastritis IL-17 correlates with the density of Hp infection, however, suggests a possible direct effect of the infection on IL-17 induction.

The Hp-associated gastritis is characterized by an infiltrate constituted by both acute and chronic inflammatory cells. The migration of PMN into the mucosa is thought to be dependent on the expression of chemokines (1, 2). Once they arrive in the mucosa, these cells become active and release enzymes that are potentially capable of causing epithelial damage (1). Several lines of evidence indicate that locally induced IL-8 plays a major role in the recruitment of PMN (3–5, 7). Although Hp by itself or its product has been reported to be a potent inducer of IL-8 synthesis, it is well known that cytokines released by activated macrophages and T cells may modulate IL-8 expression in both mononuclear and epithelial cells (9). In this context, an interesting finding of the present study is the demonstration that in the gastric mucosa, IL-17 has regulatory activity on IL-8 synthesis. Neutralization of IL-17 resulted in a significant inhibition of IL-8 production in gastric LPMC, and the addition of rhIL-17 to the cultures of MKN 28 cells increased IL-8 secretion. These data thus support previous studies indicating the ability of IL-17 to induce IL-8 expression in other systems (15). Although the mechanisms through which IL-17 enhances IL-8 have not been extensively investigated, it is known that IL-17 can activate transcription factors, such as NF-kB and mitogen-activated protein kinases (31), that are capable of initiating IL-8 expression (12, 32). We moreover showed that through selective induction of IL-8, IL-17 enhanced the in vitro chemotaxis of PMN, suggesting a possible role for this cytokine in maintaining and expanding the acute inflammatory component during Hp infection.

In addition to its ability to enhance IL-8 production, IL-17 might modulate the expression of other molecules relevant to the pathophysiology of peptic disease. Indeed, IL-17 can increase the synthesis of both proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and stromelysin-1, a matrix metalloproteinase (13, 31, 33). This enzyme is capable of causing mucosal degradation and has been associated with gastrointestinal ulceration (33). In conclusion, we report an increased expression of T cell-derived IL-17 in the gastric mucosa of patients with Hp infection, suggesting that this cytokine may play an important role in the inflammatory response to the Hp colonization and ultimately influence the outcome of the Hp-associated disease.

References


