Changes in the mucosal expression of interleukin 15 in *Helicobacter pylori*-associated gastritis

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Received 25 November 1998; accepted 18 February 1999

Abstract

Transcripts for interleukin (IL) 15 were detected in the gastric mucosal samples of 5/5 (100%) patients with no evidence of *Helicobacter pylori* infection and in 4/14 (28%) *H. pylori*-infected patients \( (P<0.05) \). Both IL-15 mRNA and IL-15 protein were detected in 1/6 (17%) patients who successfully underwent *H. pylori* eradication therapy, before treatment and in 5/6 (83%) cases after eradication. Even though a parallel significant \( (P<0.03) \) improvement of gastritis score occurred after eradication, the severity of gastritis did not differ according to the mucosal IL-15 expression among *H. pylori*-infected patients, irrespective of the CagA serology. This study demonstrates, for the first time, that transcripts for IL-15 are expressed in the human gastric mucosa. Changes occurring during *H. pylori* colonisation and after eradication raise the hypothesis that *H. pylori* may down-regulate IL-15 expression in the gastric mucosa. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: *Helicobacter pylori*; Interleukin 15; Gastritis

1. Introduction

Interleukin (IL) 15 is a novel 14–15-kDa pleiotropic cytokine expressed in different cell types, including intestinal epithelial cells and activated monocytes and macrophages. It shares two receptor subunits and a number of functional effects with IL-2 such as leukocyte recruitment and Th1 immune response regulation [1]. These observations are consistent with studies showing that IL-15 stimulates the proliferation of natural killer cells and acts as a costimulator with IL-12 to facilitate the production of interferon-\( \gamma \) by these cells [2]. However, IL-15 also induces IL-5 production from allergen-specific human T-cell clones [3] and supports B-cell proliferation and immunoglobulin synthesis in vitro [4], implying a positive role in Th2-mediated responses.
Recent experiments show that intestinal epithelial cells both express and respond to IL-15, suggesting that IL-15 may integrate intestinal epithelial cell function with the intestinal immune system [5].

The host immune and inflammatory response is emerging as the crucial element in the pathogenesis of Helicobacter pylori-associated gastro-duodenal diseases [6]. Although there is mounting evidence from many laboratories to support the hypothesis that Th2 cells are very important in bacterial clearance, studies indicate that Th1-like immune response along with chronic active leukocyte infiltration and a perturbation of cytokine networks are features of H. pylori-associated gastritis [7–9]. In this study we explored whether IL-15 is involved in the mucosal immune response during H. pylori-associated gastritis. Specific aims were: (1) to determine whether IL-15 is expressed in the human gastric mucosa; (2) to investigate whether mucosal IL-15 is dysregulated during active H. pylori gastritis; (3) to examine whether H. pylori eradication influences mucosal IL-15 expression.

2. Materials and methods

2.1. Patients and samples

Nineteen patients (12 men, 7 women; age range 18–75 years, median 42) who underwent gastroscopy (Olympus GIF V3) for dyspeptic complaints were studied; 17 had non-ulcer dyspepsia and two had duodenal ulcer. Eight patients with evidence of H. pylori infection were given a 1-week triple therapy consisting of clarithromycin (2×250 mg day⁻¹), tinidazole (2×500 mg day⁻¹), and lansoprazole (2×30 mg day⁻¹) and were re-endoscoped after 6–8 weeks. During endoscopy, eight antral and corpus biopsies were taken from each patient. Two antral and one corpus samples were used for gastric histology and one antral sample for urease quick test (Yamanouchi Pharma, Milan, Italy; 94% sensitivity, 100% specificity). The remaining two antral and two corpus samples were utilised for IL-15 analysis. To evaluate whether H. pylori could affect other cytokines produced by intestinal epithelial cells, the expression of IL-7 was further assessed in the antral and corpus mucosal samples of nine patients.

2.2. Gastric histology

Sections of biopsy specimens were stained with haematoxylin and eosin to examine gastritis and with Giemsa to detect H. pylori. The degree and activity of gastritis and the density of H. pylori colonisation, atrophy, and intestinal metaplasia were graded according to the Sydney system [10] on a 4-point scale: no (0), mild (1), moderate (2), and severe (3) changes.

2.3. H. pylori status

In order to avoid false-negative and false-positive findings, untreated and treated patients were classified as H. pylori-positive or -negative when both the urease quick test and Giemsa-stained sections in either antral and/or corpus gastric samples were concordantly positive or negative. H. pylori eradication was further assessed by means of the urea C13 breath test (Cortex, Italy) as it is especially valuable for following up patients after therapy (98% sensitivity, 95% specificity; personal data).

2.4. Serologic assays

Serum samples from each patient were tested for IgG antibodies to H. pylori (Helori-IgG, Eurospital, Italy; 94% sensitivity, 84% specificity) and to the cytotoxin-associated gene (CagA) (Helori-CTX IgG, Eurospital, Italy; 94.1% sensitivity, 97.9% specificity) by enzyme-linked immunosorbent assay (ELISA).

2.5. Gastric tissue homogenate preparation for RNA analysis

Gastric biopsy specimens were used for RNA analysis on freshly obtained whole tissue. One biopsy was taken from the antrum and one from the corpus of each patient. Antral and corpus biopsy specimens were separately placed in guanidine thiocyanate buffer on ice, homogenised using a tissue homogeniser (Ystral GmbH, PBI International, Dottingen, Germany) and immediately used for RNA extraction.

2.6. RNA and complementary-DNA preparation

Total RNA was extracted from mucosal samples
according to Chomczynsky and Sacchi [11]. The sample obtained was quantitated by absorbance at 260 nm. RNA integrity was assessed by electrophoresis on a 1.5% agarose gel. Complementary DNA (cDNA) was synthesised from 0.5 \( \mu \)g of total RNA using 0.2 U of murine leukaemia virus reverse transcriptase (Promega, Madison, WI, USA), 2.5 \( \mu \)M random hexamers (Boehringer-Mannheim, Mannheim, Germany), 1 mM dNTP (Boehringer-Mannheim), 2 U RNase inhibitor (Promega) in a total volume of 20 \( \mu \)l. The reaction was performed at 37°C for 60 min.

2.7. Reverse-transcription polymerase chain reaction

Prior to examining transcripts for IL-15 (200 bp) and IL-7 (323 bp), sample cDNA content was normalised on \( \beta \)-actin (485 bp) signal. For this purpose, 5 \( \mu \)l of cDNA was incubated in a reverse-transcription polymerase chain reaction (RT-PCR) for 19, 21, 23, 25 and 35 cycles with specific \( \beta \)-actin primers. IL-15 and IL-7 primers were assayed on all samples by incubating the same amount of cDNA for 35 cycles. PCR reactions were performed in a total volume of 50 \( \mu \)l in the presence of 1 U of Taq DNA Polymerase (Boehringer-Mannheim), 200 \( \mu \)mol dNTPs (Boehringer-Mannheim) and 25 pmol \( l^{-1} \) 5' and 3' primers. Reactions were incubated in a Robocycler thermal cycler (Stratagene, La Jolla, CA, USA) (denaturation 1 min at 94°C; annealing for 1 min at 57°C for both \( \beta \)-actin and IL-15 and for 1 min at 55°C for IL-7; extension for 1 min at 72°C). PCR primers (Genosys, Cambridge, UK) were as follows: IL-15: 5'-GTATTGTAGGAGGCATCGTG-3'; 3'-GAAGCTGGCATTCATGTCTTC-5'; IL-7: 5'-GGACTACCACATGAACTCTG-3'; 3'-CATATCTTGCCCAAGTGTTAC-5'; \( \beta \)-actin: 5'-CGAGGCCAGAGCAAGAGA-3'; 3'-CTGTGACATTAAGGAGAGCT GTG-5'.

In order to exclude the amplification of genomic DNA contaminating the samples, we used primers derived from separate exons and experiments were also performed using RNA as substrate for PCR assay. 10 \( \mu \)l PCR product was combined with 1 \( \mu \)l of loading buffer and electrophoresed on a 1.5% agarose gel (in Tris-ethylenediaminetetraacetic acid buffer). A 123-bp ladder was used to assess sample size. The specificity of the PCR products for IL-15 was confirmed by restriction enzyme analysis. For this purpose the amplified IL-15 product was digested with \( RsaI \) (Boehringer-Mannheim) into two fragments of 116 and 82 bp.

2.8. IL-15 enzyme-linked immunosorbent assay

To examine whether IL-15 was produced in the human gastric mucosa, one antral biopsy specimen was taken from the eight patients who underwent \( H. pylori \) eradication therapy. Samples were weighed and immediately placed in 1 ml lysis buffer containing 10 \( \mu \)g ml\(^{-1} \) aprotinin (Sigma Chemicals, St. Louis, MO, USA), and homogenised on ice. After running at 3000 \( \times g \) for 30 min at 4°C, supernatants were collected and stored at -80°C until assay. Samples were normalised for tissue weight before testing IL-15. IL-15 was measured using a sensitive ELISA (Genzyme Corporation, Cambridge, MA, USA). According to the manufacturer’s directions the minimum detectable IL-15 concentration was 10 pg ml\(^{-1} \).

2.9. Statistics

Data are presented as mean values with standard deviation (S.D.). Frequencies were compared by means of Fisher’s exact test. The Wilcoxon signed rank test was used with paired data, and the Mann-Whitney \( U \)-test with unpaired data. A \( P \) value of less than 0.05 was considered significant.

3. Results

Fourteen out of the 19 (73%) dyspeptic patients had evidence of \( H. pylori \) infection. Eight out of these 14 (57%) \( H. pylori \)-infected patients tested positive for IgG antibodies to CagA. Five patients had no evidence of \( H. pylori \) infection by either biopsy-based or serological tests. Transcripts for IL-15 were detected in all patients with no evidence of \( H. pylori \) infection (5/5, 100%) and in four out of the 14 (28%) \( H. pylori \)-infected patients (\( P < 0.05 \)). In each patient, the finding of IL-15 mRNA did not differ between antral and corpus biopsy samples. Six patients successfully underwent \( H. pylori \) eradication therapy: both IL-15 mRNA and IL-15 protein (35 pg ml\(^{-1} \)) were detected in only one case before treat-
ment and in five cases (60 ± 33 pg ml⁻¹) after eradication. Fig. 1 shows that in four cases IL-15 mRNA was not detected before treatment while it was detectable after eradication of H. pylori, in four patients. Transcripts for β-actin (485 bp) were detected in all cases, before treatment and after eradication of H. pylori.

![Fig. 1. Determination of IL-15 (200 bp) expression in homogenised biopsy specimens from gastric mucosa by reverse-transcription PCR. Samples were taken from six patients before treatment and after eradication of H. pylori. Lane 1, 123-bp ladder. Lanes 4-7, transcripts for IL-15 were undetectable before treatment while they were detected after eradication of H. pylori, in four patients. Transcripts for β-actin (485 bp) were detected in all cases, before treatment and after eradication of H. pylori.](https://academic.oup.com/femspd/article-abstract/24/2/233/758061)

### Table 1
Gastritis scores in six dyspeptic patients before treatment and after eradication of H. pylori

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>6.0 ± 0.9*</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.2 ± 1.6*</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>Sum score</td>
<td>10.2 ± 2*</td>
<td>3.2 ± 1.9</td>
</tr>
</tbody>
</table>

The severity of gastritis was graded according to the Sydney system [10]. Data are given as mean ± S.D. *P<0.03, compared to data after eradication.

### Table 2
Gastritis scores among the 14 patients with evidence of H. pylori infection according to the expression of IL-15

<table>
<thead>
<tr>
<th>IL-15-positive (n = 4)</th>
<th>IL-15-negative (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>6.2 ± 0.9*</td>
</tr>
<tr>
<td>Corpus</td>
<td>3.5 ± 3.3*</td>
</tr>
<tr>
<td>Sum score</td>
<td>9.7 ± 2.4*</td>
</tr>
</tbody>
</table>

The severity of gastritis was graded according to the Sydney system [10]. Data are given as mean ± S.D. *P<0.05, compared to data from IL-15-negative patients.

*Two (50%) patients tested positive for CagA serology.

*Six (60%) patients tested positive for CagA serology.

### 4. Discussion

In recent years, attention in the pathogenesis of infectious diseases has focused on the principal mediators which orchestrate the inflammatory response, namely cytokines. Furthermore, it has become apparent that bacteria produce many molecules which deeply affect the capacity of leukocytes and tissue cells to produce selected cytokine networks [12]. Infection with H. pylori is associated with an abundant inflammatory response and epithelial cell injury in human gastric mucosa which may lead to peptic ulcer, gastric lymphoma and carcinoma as opposed to patients with gastritis alone. The host response to natural infection, perhaps triggered by specific strains or regulated by specific host factors, may be inappropriate to clear the organism thus contributing to the spectrum of H. pylori-associated diseases. It has been proposed that an excessive Th1 response driven by H. pylori, through the production of the specific cytokines, increases inflammation and epithelial damage and may account for the persistence of the infection [6]. In fact, a definite Th2 response which seems to be more important in the generation of mucosal secretory immune responses and protective immunity has not been documented in H. pylori-associated gastritis [6]. Moreover, protection against extracellular bacteria (i.e. H. pylori) is widely thought to depend mainly on neutrophils and antibodies [13].

IL-15 is a pleiotropic cytokine which exerts different effects but whether it prejudices Th1 or Th2 differentiation is unclear [14]. This study demonstrated, for the first time, that transcripts for IL-15 are expressed in the human gastric mucosa and that IL-15
changes occur during gastric *H. pylori* colonisation. It was also documented that gastric mucosa IL-15 mRNA was associated with detectable levels of IL-15 protein in the same material. Whether the gastric epithelial cells or the lamina propria mononuclear cells are the source of IL-15 cannot be ascertained from our experiment. However, according to other experiments dealing with the colonic mucosa [5], it can be presumed that both cell types are able to express and release IL-15. In our series, IL-15 mRNA was detected in the gastric mucosa of all patients with no evidence of *H. pylori* infection as assessed by means of biopsy-based tests and further corroborated by serology to exclude the possibility of a past infection with *H. pylori*. Since IL-15 is considered an important factor in the innate immune response, this finding was not unexpected. However, when patients infected with *H. pylori* were examined, it was found that mostly (72% of cases) IL-15 mRNA was not expressed in their gastric mucosa. Furthermore, patients with successful eradication of *H. pylori* mostly (80% of cases) displayed a restoration of the IL-15 pool, with both transcript and protein found in their gastric mucosal samples. At the same time, IL-7, a regulatory cytokine produced by macrophage-like cells, including intestinal epithelial cells, was invariably detected in all tested samples, irrespective of the *H. pylori* status. Eradication of *H. pylori* was associated with a significant improvement of gastritis score along with the restoration of mucosal IL-15. However, among *H. pylori*-infected patients, gastritis score did not differ according to the presence of IL-15, suggesting that IL-15 does not actually parallel the severity of gastritis. Furthermore, it was found that CagA serology did not affect IL-15 relationship with gastritis score.

The role of IL-15 in the host defence against bacterial infections is not yet elucidated. It could offer a contribution to the early phase of immune responses, providing enhancement of polymorphonuclear and natural killer cell responses, and subsequently T-cell responses, prior to optimal IL-2 production [14]. The main function of activated polymorphonuclear cells is to phagocytose and kill microbial pathogens. Production of IL-15 in the early steps of the inflammatory response to pathogens might increase the number of polymorphonuclear cells infiltrating the tissues.

The findings of this preliminary study raise the hypothesis that *H. pylori* may down-regulate IL-15 expression so interfering with the innate immune gastric mucosal response to the bacterium. Further experiments are needed to confirm whether IL-15 is involved in the protection against *H. pylori* and to explore the interrelationship between IL-15 and Th1/Th2 cytokine networks underlying the host response to *H. pylori* infection.

Acknowledgements

This work was supported by Grant CNR 97.04550.CT04 from the Italian National Research Council.

References


