Evidence for the role of gastric mucosa at the secretion of soluble triggering receptor expressed on myeloid cells (strem-1) in peptic ulcer disease

Vassilios Koussoulas, Spyridon Vassiliou, Ekaterini Spyridaki, Maria Demonakou, Ilia Vaki, Charalampos Barbatzas, Helen Giamarellou, Evangelos J Giamarellos-Bourboulis

Abstract

AIM: To investigate the role of gastric mucosa at the secretion of stREM-1 in peptic ulcer.

METHODS: Seventy-two patients were enrolled; 35 with duodenal, 21 with gastric ulcer and 16 with chronic gastritis. Patients were endoscoped and gastric juice was aspirated. Patients with duodenal and gastric ulcer underwent a second endoscopy post-treatment. Biopsies were incubated in the absence/presence of endotoxins or gastric juice. Supernatants were collected and stREM-1 and TNFα were measured by enzyme immunoabsorbent assays. Scoring of gastritis was performed before and after treatment according to updated Sydney score.

RESULTS: Patients with duodenal and gastric ulcer and those with chronic gastritis had similar scores of gastritis. stREM-1 was higher in supernatants of tissue samples of H pylori-positive than of H pylori-negative patients with gastric ulcer. Median (± SE) stREM-1 was found increased in supernatants of patients with gastric ulcer before treatment (203.21 ± 88.91 pg/1000 cells) compared to supernatants either from the same patients post-treatment (8.23 ± 5.79 pg/1000 cells) or from patients with chronic gastritis (6.21 ± 0.71 pg/1000 cells) (P < 0.001 and < 0.001, respectively). Similar differences for stREM-1 were recorded among LPS-stimulated tissue samples of patients (P = 0.001). Similar differences were not found for TNFα. Positive correlations were found between stREM-1 of supernatants from patients with both duodenal and gastric ulcer before treatment and the degree of infiltration of neutrophils and monocytes.

CONCLUSION: stREM-1 secreted by the gastric mucosa is an independent mechanism connected to the pathogenesis of peptic ulcer. stREM-1 was released at the presence of H pylori from the inflamed gastric mucosa in the field of gastric ulcer.

© 2007 WJG. All rights reserved.

Key words: stREM-1; Chronic gastritis; Gastric ulcer; Duodenal ulcer

INTRODUCTION

Many aspects of the mechanisms implicated in the pathogenesis of peptic ulcer disease remain unclear[3]. Research has been focused more on derangements of mechanisms of protection and repair of the mucosa of the stomach and duodenum[3]. It appears that most cases of both gastric and duodenal ulcer occur in the setting of infection by H pylori. Evidence is mounting in support of H pylori as a necessary ingredient in the ulcerative process, similar to acid and pepsin. It is not known whether the bacteria or the accompanying inflammation is the most important factor in the pathophysiology of peptic ulcer disease[3].

Triggering receptor expressed on myeloid cells (TREM)-1 is a recently discovered receptor expressed on the surface of neutrophils and monocytes. Engagement of TREM-1 has been reported to stimulate the synthesis of proinflammatory cytokines[4]. A soluble form of TREM-1, named stTREM-1, was observed and identified at significant levels in serum samples of patients with diseases affecting the gastrointestinal tract[5].

stREM-1 has been found elevated in the gastric juice
of patients with peptic ulcer. Since its levels were positively correlated to the degree of infiltration of the gastric mucosa by neutrophils, thus to the hypothesis that sTREM-1 might be a sign of an inflammatory reaction taking place in the gastric mucosa. The latter hypothesis might be strengthened by the lack of expression of TREM-1 on cell membranes of macrophages of healthy intestinal lamina propria.

Based on the latter evidence, it is questioned whether sTREM-1 might be produced from the gastric mucosa in the event of peptic ulcer disease. The current study was designed to investigate a role of gastric mucosa for the release of sTREM-1. Furthermore it was investigated whether anti-ulcerative treatment was accompanied by any change of the ability of the mucosa for the secretion of sTREM-1.

MATERIALS AND METHODS

Study group
The study was approved by the Medical and Ethics Committee (6th/11.30.05/26 962) of General Hospital “Sismanoglion”, Athens, Greece. A total of 72 patients, 54 men and 18 women, aged 58.92 ± 16.52 (mean ± SD) years were enrolled; 56 patients with peptic ulcer disease and 16 patients with chronic gastritis without peptic ulcer. Admitted patients were divided into three groups based on endoscopic findings, as follows: group A: consisting of 35 patients with duodenal ulcer; group B: consisting of 21 patients with gastric ulcer; and group C, consisting of 16 patients with chronic gastritis.

Informed consent was obtained from all participants. Indications for endoscopy in these patients were abdominal pain or discomfort, epigastric pain with nausea and vomiting, and dyspepsia. All endoscopies were done by the same endoscopist. Peptic ulcer was defined as a circumscribed break in the mucosa in the duodenum (DU) or in the stomach (GU) with apparent depth covered by an exudate, as previously described. All patients with peptic ulcer disease belonged to the Forrest III score. H. pylori infection was defined by the presence of the bacterium both at the histopathologic findings of each biopsy and after a gastric biopsy culture at the proper growth medium. Exclusion criteria for the study were: recent upper GI bleeding, gastric carcinoma, diabetes mellitus, liver cirrhosis, acute or chronic renal failure and the ingestion of any medication and the second 15 d after the end of the treatment. Among patients with chronic gastritis only one endoscopy was done; at each endoscopy biopsies were collected. Gastric juice was aspirated immediately after the entrance of the endoscope into the gastric lumen. At the time of endoscopy, three biopsy specimens were obtained from adjacent areas of the gastric antrum. When each biopsy specimen was taken, the forceps were fully opened and aimed at right angles to the gastric lumen to the extent possible to obtain uniformly sized biopsies. Biopsies were obtained from endoscopically intact mucosa distant from focal lesions such as ulcers and erosions. Each biopsy was used for in vitro culture.

After diagnosis of peptic ulcer disease or gastritis, omeprazole 20 mg twice daily was prescribed. It was administered for four weeks in patients with duodenal ulcers, for eight weeks in patients with gastric ulcers and for four weeks in patients with chronic gastritis. For patients with infection by H. pylori, clarithromycin 500 mg bid and amoxicillin 1000 mg bid for 10 d were also added. The above treatment was administered according to international guidelines. In brief, gastric antral mucosal biopsy tissues were weighed and cultured on a culture insert over wells containing RPMI 1640 medium with 10% heat-inactivated fetal bovine serum in a 5% CO2 incubator for 18 h. Biopsies were positioned on the insert with mucosal surfaces up. The first biopsy tissue was left unstimulated and served as control; the second was stimulated with 10 ng/mL of lipopolysaccharide of Escherichia coli O144:H4 (LPS), and the third with 30 μL of gastric juice of each patient. The total volume of the added growth medium was 2.4 mL; when gastric juice was added it represented 1.25% of the total well volume. At the end of the incubation, the plates were centrifuged for ten minutes at 1400 g; then supernatants were collected from the wells and stored at -70°C, until assayed for estimation of sTREM-1 and TNFα. Results were correlated with histopathological findings.

Estimation of sTREM-1
Estimation of sTREM-1 was performed by a homemade enzyme immunoabsorbent assay in samples of supernatants. Capture antibody of sTREM-1 (R&D Inc, Minneapolis, USA) was diluted to 4000 ng/mL and distributed in a 96-well plate at a volume of 0.1 mL per well. After overnight incubation at 25°C, wells were thoroughly washed with a 0.05% solution of Tween in PBS (Merck) (pH: 7.2-7.4). Then 0.1mL of standard concentrations of sTREM-1 (15.1-4000 pg/mL, R & D Inc) diluted with Reagent diluent (1% BSA in PBS, pH 7.2-7.4, 0.2 micron filtered) serving as a buffer or of supernatants was added in wells. After incubation for two hours, wells were washed thrice, and 0.1 mL of one 400 ng/mL dilution of sTREM-1 detection antibody (R&D Inc) was added per well. The plate was then incubated for two hours, and attached antibodies were signalled by streptavidin. Concentrations of sTREM-1 to each well were estimated by the optical density detected at 450 nm after addition of one 1:1 solution of H2O2: tetramethylbenzidine as a substrate (R&D Inc). sTREM-1 was expressed in pg/g of tissue. The lowest limit of detection for sTREM-1 was 3.91 pg/g of tissue. All determinations were performed in duplicate; the inter-day variation of the assay was 5.23%.

Estimation of TNFα
Tumor necrosis factor alpha (TNFα) was measured in samples of supernatants with an enzyme immunoabsor-
bent assay (EIA, Amersham, London, UK). Lowest limits of detection were 6.25 pg/g of tissue. All measurements were performed in duplicate and cytokine concentrations were expressed as pg/g of tissue.

**Histopathology**

Formalin-fixed, paraffin-embedded tissue samples were routinely cut at 3-4 μm and stained with haematoxylin and eosin alcan blue PAS (Periodic Acid Schiff) (at pH: 2.5) and Giemsa. Immunohistochemistry was performed for *H pylori* detection with 1:100 dilution (Biocare Med., California, USA).

The presence of gastritis was evaluated in each biopsy sample after separate scoring for each of the following parameters: (a) disease activity as mucosal infiltration by neutrophils; (b) chronic inflammation expressed as infiltration by monocytes and lymphocytes; (c) degree of mucosal atrophy; and (d) intestinal metaplasia. Each parameter was scored from 0 to 3 (0: absent; 1: mild; 2: moderate; 3: marked). In the case of intestinal metaplasia scores indicated the following findings: 0: absence; 1: complete or type I; 2: incomplete or type II; and 3: incomplete or type III. As a consequence total Sydney score of gastritis either without or with stimulation by LPS (P < 0.05). Concentrations of sTREM-1 were also higher in supernatants of gastric mucosa taken from patients with duodenal ulcer disease or gastric ulcer disease and chronic gastritis for comparisons < 0.01 and < 0.01, respectively). No changes were recorded between patients with either duodenal ulcer disease or gastric ulcer disease or chronic gastritis either without or with stimulation by LPS (P < 0.05). The presence of *H pylori* was evaluated in a subpopulation of patients with duodenal ulcer disease and chronic gastritis for comparisons < 0.01 and < 0.01, respectively). Similar differences were not found for TNFα. They were also not found for both sTREM-1 and TNFα between *H pylori*-positive and *H pylori*-negative patients with gastritis.

In the above subgroups of patients, concentrations of sTREM-1 were also higher in supernatants of gastric mucosa of *H pylori*-positive patients with gastritis than of mucosa of *H pylori*-positive patients with duodenal ulcer disease after stimulation by LPS (P < 0.05). Concentrations of sTREM-1 were also higher in patients with duodenal ulcer disease compared to gastric ulcer disease or chronic gastritis for comparisons < 0.01 and < 0.01, respectively). Similar differences were not found between *H pylori*-negative patients with gastritis and patients with chronic gastritis.

### Statistical analysis

Patients of three groups were further divided into subgroups according to the presence or absence of *H pylori*. Concentrations of sTREM-1, and TNFα were expressed by their median ± 95% confidence intervals (CI) or range. For qualitative data comparisons were performed by χ² test. Correlations between sTREM-1, and TNFα and the gastritis score or the density of *H pylori* were performed according to Spearman’s rank of order. Any P value less than 0.05 was considered as significant.

### RESULTS

Patients’ characteristics are given in Table 1. All patients suffering from duodenal ulcers had presence of *H pylori* on tissue biopsy.

No differences were recorded between patients with duodenal and gastric ulcer disease before treatment and patients with chronic gastritis regarding histological parameters of gastritis according to updated Sydney score. Differences in total updated Sydney score, and several parameters of chronic gastritis before and after treatment among patients with peptic ulcer disease and patients with chronic gastritis are shown in Table 1.

Concentrations of sTREM-1 and of TNFα in supernatants of samples of gastric mucosa taken from patients with either duodenal ulcer disease or gastric ulcer disease or chronic gastritis for comparisons < 0.01 and < 0.01, respectively). Similar differences were not found for TNFα. They were also not found for both sTREM-1 and TNFα between *H pylori*-positive and *H pylori*-negative patients with chronic gastritis.
Table 2 Concentrations of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and tumor necrosis factor-alpha (TNFα) of supernatants of tissue samples taken from patients with either duodenal or gastric ulcer disease or chronic gastritis at pre-treatment

| Parameters                  | Duodenal ulcer | Gastric ulcer | Chronic Gastritis |
|-----------------------------|----------------|---------------|-------------------|
|                             | H pylori (+)   | H pylori (-)  | H pylori (+)      | H pylori (-)   |
| N                           | 35             | 0             | 14                | 9               |
|                             | sTREM-1 [median (range), pg/g of tissue] |             |                   |                   |
| 0                           | -              | 317.27 (73.98) | 112.41 (38.71)   | 6.21 (0.81)    |
| LPS                         | -              | 455.41 (98.53) | 214.55 (44.81)   | 12.67 (2.16)   |
| Gastric juice               | 17.91 (7.11)   | 118.48 (54.93) |                   | 10.07 (2.02)   |
| Presence of LPS             | -              | 6.40 (1.52)    |                   | 7.42 (1.22)    |
|                             | 7.58 (1.75)    | 21.44 (8.12)   |                   | 8.11 (1.31)    |
| LPS                         | 28.25 (3.35)   | 144.90 (58.79) |                   | 19.07 (4.72)   |
| Gastric juice               | 18.02 (5.97)   | 86.82 (35.41)  |                   | 17.87 (2.11)   |

Patients were divided as either H pylori-positive or H pylori-negative. LPS: Endotoxin of Escherichia coli O144:H4. *P < 0.05, **P < 0.01, vs H pylori negative patients. **P vs H pylori negative patients. non-significant.

Table 3 Influence of treatment on the secretion of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and tumor necrosis factor-alpha (TNFα) by the mucosa of H pylori-positive patients with gastric and duodenal ulcers

| Parameters                  | Gastric ulcer | Duodenal ulcer |
|-----------------------------|---------------|----------------|
|                             | Pre-treatment | Post-treatment |
| H pylori-positive with gastric ulcer | sTREM-1 [median (range), pg/g of tissue] | 8.23 (5.79) |
|                             | 203.21 (88.91) | 8.23 (5.79) |
| LPS                         | 418.87 (127.56) | 14.31 (7.11) |
| Presence of LPS             | 12.67 (226.79) | 12.47 (20.41) |
|                             | 31.07 (231.89) | 34.05 (150.36) |

Table 4 Correlations between sTREM-1 and TNFα and parameters of gastritis score in non and LPS stimulated supernatants of H pylori positive patients with duodenal and gastric ulcer and of patients with chronic gastritis

| Parameters of Gastritis score | Supernatants of mucosa sampled from H pylori-positive patients with duodenal ulcer disease |
|------------------------------|------------------------------------------------------------------------------------------|
|                              | Absence of LPS | Presence of LPS |
|                              | sTREM-1 | TNFα | sTREM-1 | TNFα |
| Neutrophils infiltration     | P < NS  | P < NS | P < NS  | P < NS |
| Monocytes infiltration       | P < NS  | P < 0.01 | P < 0.01 | P < 0.01 |
| Supernatants of mucosa sampled from H pylori-positive patients with gastric ulcer disease | Pre-treatment |
| Neutrophils infiltration     | P < NS  | P < 0.05 | P < 0.05 | P < 0.05 |
| Monocytes infiltration       | P < 0.01 | P < 0.01 | P < 0.01 | P < 0.01 |
| Supernatants of mucosa sampled from H pylori-positive patients with chronic gastritis | Pre-treatment |
| Neutrophils infiltration     | P < NS  | P < 0.05 | P < NS  | P < 0.05 |
| Monocytes infiltration       | P < NS  | P < 0.05 | P < NS  | P < 0.05 |

Among patients suffering from chronic active gastritis only a minority evolves to peptic ulcer disease, rendering the question what might be the underlying mechanism leading several patients with gastritis to peptic ulcer and others not. Recent data revealed that sTREM-1 was found increased in the gastric juice of patients with peptic ulcer disease compared to patients with chronic gastritis. That finding might lead to the hypothesis that sTREM-1 might constitute an independent factor leading from gastritis to peptic ulcer.

The present study applied a unique design. It is the first time, to our knowledge, in the literature where the ability of
the gastric mucosa for the release of sTREM-1 and TNFα was studied among patients with either duodenal ulcer or gastric ulcer or chronic gastritis without signs of peptic ulcer disease. Supernatants of biopsies taken from the enrolled patients were stimulated with LPS and gastric juice of patients. It is known that H pylori-as a Gram negative bacterium-secretes LPS that mediates to the gastric inflammation[6]. As described by others, cell loss and apoptosis of gastric mucous cells was enhanced by H pylori LPS with less potency compared to the same effect by Es. coli LPS. The low immunological potency of H pylori LPS may contribute to low-grade gastritis[16]. In an attempt to simulate the latter process cultured biopsies were stimulated with LPS. Inflammation of the gastric mucosa was significantly reduced after treatment whereas H pylori was also eradicated (Table 1). Although the proper treatment was administered in patients with chronic gastritis second endoscopy was not performed; the latter was thought to be of no significance because sTREM-1 concentrations were already minimal pre treatment.

Results revealed that gastric mucosa of H pylori positive patients with both duodenal and gastric ulcer disease was potent to secrete sTREM-1. The potency for secretion of sTREM-1 was lost post-treatment. The release of sTREM-1 was higher by H pylori infected gastric mucosa than by gastric mucosa not infected by H pylori. The effect of H pylori on the release of sTREM-1 by mucosa of patients with duodenal ulcer could not be assessed since all patients with duodenal ulcer in the studied cohort were H pylori-positive.

Similar kinetics to sTREM-1 were not found for TNFα. TNFα was found increased in strict correlation with the degree of mucosal inflammation independently from the underlying pathogenetic status. The latter was highlighted by the fact that TNFα was increased post treatment when gastric mucosa was stimulated by LPS (Table 4).

The main assumption revealed from the presented study was that sTREM-1 was secreted by the activated inflammatory cells that infiltrate the gastric mucosa; inflammatory cells were immigrated to the inflamed gastric mucosa attracted by H pylori or its components. The treatment of inflamed gastric mucosa and the eradication of H pylori ceased the secretion of sTREM-1. It is of great importance that the latter assumption exists only in the status of gastric and duodenal ulcer disease. The release of sTREM-1 was independent from the density of mucosal inflammation at patients with no evidence of peptic ulcer. The pattern of release of sTREM-1 by the activated inflammatory cells and probably their intracellular activity should be further investigated.

The independent contribution of sTREM-1 release in the pathogenesis of gastric ulcer is further aggravated by the observation that gastric juice could not influence the activity of the inflamed mucosa per se. Stimulation of inflamed gastric mucosa with gastric juice was not lead to a significant increase to the release of sTREM-1 and TNFα (Table 4).

The present study revealed for the first time in the literature that sTREM-1 secreted by the gastric mucosa is an independent mechanism connected to the pathogenesis of gastric and duodenal ulcer. sTREM-1 was released at the presence of H pylori from the inflamed gastric mucosa in the field of gastric ulcerative process. The exact pathogenetic mechanisms needs to be further clarified.

REFERENCES

1. Padol S, Yuan Y, Thabane M, Padol IT, Hunt RH. The effect of CYP2C19 polymorphisms on H. pylori eradication rate in dual and triple first-line PPI therapies: a meta-analysis. Am J Gastroenterol 2006; 101: 1467-1475
2. Calam J, Baron JH. ABC of the upper gastrointestinal tract: Pathophysiology of duodenal and gastric ulcer and gastric cancer. BMJ 2001; 323: 980-982
3. Eckmann L. Sensor molecules in intestinal innate immunity against bacterial infections. Curr Opin Gastroenterol 2006; 22: 95-101
4. Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. N Engl J Med 2004; 350: 451-458
5. Tzivras M, Koussoulas V, Giamarellous-Bourboulis EJ, Tzivras D, Tsaganos T, Koutoukas P, Giamarellou H, Archimandritis A. Role of soluble triggering receptor expressed on myeloid cells in inflammatory bowel disease. World J Gastroenterol 2006; 12: 3416-3419
6. Koussoulas V, Vassiliou S, Demonakou M, Tassias G, Giamarellous-Bourboulis EJ, Mouktaroudi M, Giamarellou H, Barbatzas C. Soluble triggering receptor expressed on myeloid cells (sTREM-1): a new mediator involved in the pathogenesis of peptic ulcer disease. Eur J Gastroenterol Hepatol 2006; 18: 375-379
7. Schenk M, Bouchon A, Birrer S, Colonna M, Mueller C. Macrophages expressing triggering receptor expressed on myeloid cells-1 are underrepresented in the human intestine. J Immunol 2005; 174: 517-524
8. Peek RM, Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, Blaser MJ. Heightened inflammatory response and cytokine expression in vivo to cagA+ Helicobacter pylori strains. Lab Invest 1995; 73: 760-770
9. Kohler B, Maier M, Benz C, Riemann JF. Acute ulcer bleeding. A prospective randomized trial to compare Doppler and Forrest classifications in endoscopic diagnosis and therapy. Dig Dis Sci 1997; 42: 1370-1374
10. Peterson WL, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease guidelines for testing and treatment. Arch Intern Med 2000; 160: 1285-1291
11. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996; 20: 1161-1181
12. Kusugami K, Fukatsu A, Tanimoto M, Shinoda M, Haruta J, Kuroiwa A, Ina K, Kanayama K, Ando T, Matsuura T. Elevation of interleukin-6 in inflammatory bowel disease. J Gastroenterol 2005; 40: 949-959
13. Soll AH. Consensus conference. Medical treatment of peptic ulcer disease. Practice guidelines. Practice Parameters Committee of the American College of Gastroenterology. JAMA 1996; 275: 622-629
14. Ahmed N, Sechi LA. Helicobacter pylori and gastroduodenal pathology: new threats of the old friend. Ann Clin Microbiol Antimicrob 2005; 4: 1
15. Zhang C, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. Comparison of Helicobacter pylori infection and gastric mucosal histological features of gastric ulcer patients with chronic gastritis patients. World J Gastroenterol 2005; 11: 976-981
16. Lindholm C, Quiding-Järbrink M, Lönnroth H, Hamlet A, Svennerholm AM. Local cytokine response in Helicobacter pylori-infected subjects. Infect Immun 1998; 66: 5964-5971
17. Vakil N. Helicobacter pylori treatment: a practical approach. Am J Gastroenterol 2006; 101: 497-499

S- Editor Liu Y  L- Editor Rampone B  E- Editor Li JL