INTERLEUKIN 12 DRIVES TH1 SIGNALING PATHWAY IN HELICOBACTER PYLORI-INFECTED HUMAN GASTRIC MUCOSA

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Key words: interleukin 12, T helper 1, transcription factors, interferon γ, Helicobacter pylori
Abstract

This study examines mechanisms that regulate T helper lymphocyte (Th) 1 commitment in Helicobacter pylori-infected human gastric mucosa. Interferon (IFN)-γ, interleukin (IL)-4, and IL-12 were measured in total extracts of gastric biopsies taken from H. pylori-infected and uninfected patients by enzyme linked immunosorbent assay. STAT4, STAT6, and T-bet were examined in total proteins extracted from gastric biopsies by Western blotting. Finally, the effect of a neutralizing IL-12 antibody on the expression of Th1 transcription factors and levels of IFN-γ was examined in organ cultures of H. pylori-infected biopsies. Increased levels of IFN-γ and IL-12 were found in gastric biopsy samples of H. pylori-infected patients compared to uninfected patients. Accordingly, H. pylori-infected biopsies exhibited high expression of phosphorylated STAT4 and T-bet. Higher levels of IFN-γ and expression of Th1 transcription factors were associated with higher infiltration of mononuclear cells in the gastric mucosa. By contrast, production of IL-4 and expression of phosphorylated STAT6 were not associated with the intensity of mononuclear cell infiltration. In ex vivo organ cultures of H. pylori-infected biopsies, neutralization of endogenous IL-12 down-regulated the expression of p-Stat4 and T-bet and reduced IFN-γ production. Data indicate that IL-12 contributes to the Th1 cell commitment in H. pylori-infected human gastric mucosa.
Introduction

*Helicobacter pylori* is a gram-negative extracellular organism that colonizes the gastric mucosa of humans causing chronic gastric inflammation, peptic ulcer, gastric adenocarcinoma and lymphoma. Despite the development of immune responses against *H. pylori* infection, the bacteria are rarely eliminated and colonization is generally persistent. Factors that contribute to the failure of the immune response to clear the organism are not yet fully elucidated (3).

Chronic gastric inflammation induced by *H. pylori* is considered a T-helper lymphocyte (Th)1-mediated process characterized by increased production of interferon (IFN)-γ which is implicated in perpetuating the inflammatory changes that lead to disease (7,10). Several studies have clearly demonstrated that distinct cytokine activated signaling and transcription factors regulate the commitment of naïve T cell along the Th1 or Th2 phenotype, as well as maintenance of the polarized phenotype (16). Transcription factors such as signal transducer and activator of transcription (STAT4) is associated with IFN-γ production and play a major role in Th1 specific cytokine production (22). Molecular analysis of transcription factors expressed in polarized lymphocytes has led to the identification of the novel transcription factor T-box expressed in T cells (T-bet). T-bet has been shown to be required for Th1 differentiation and its expression is sufficient to induce IFN-γ production in Th cells (24). On the other side, Th2 commitment is characterized by the activation of STAT6 and IL-4 production (28).

Cytokines involved in inducing and regulating Th1 responses have been extensively investigated and often shown redundant due to several signaling pathways that are potentially implicated in their production. Nevertheless, factors that regulate Th1 transcription factors in human gastric mucosa have not been investigated.

Interleukin (IL)-12 is a heterodimeric cytokine produced by antigen-presenting cells
that promotes the development of Th1 cells and stimulates proliferation, cytolytic activity, and IFN-γ production by T and natural killer cells (5,14). The effects of IL-12 are mediated through IL-12 receptor (R) consisting of two subunits IL-12Rβ1 and IL-12Rβ2 (19). IL-12Rβ2, that transmits the signals inside the cell, is selectively expressed on Th1 cells (21,20). Binding of IL-12 to its receptor leads to phosphorylation of STAT4 (2) and IFN-γ production.

The aim of this study was to examine mechanisms which regulate Th1 commitment in human gastric mucosa infected by *H. pylori*.
Materials and Methods

Patients and Samples
Twenty patients (12 men and 8 women; age range 19-68 years; median, 36 years) who underwent esophagogastroduodenoscopy for dyspeptic symptoms were studied. No patient had previously undergone anti-\textit{H. pylori} treatment or had received antibiotics within the previous two months. Patients were classified as \textit{H. pylori}-infected (n = 10) when at least two of the following three tests were positive: urease quick test (Yamanouchi Pharma, Milan, Italy), histology, and $^{13}\text{C}$-urea breath test (Cortex, Milan, Italy). All the three tests were required to be negative to classify patients as uninfected (n = 10). During endoscopy, 5-11 biopsies were taken in the antral area 2 cm below the incisura angularis: 1 for urease quick test, 2 for histologic examination, 2 for protein extraction, and 6 of 10 \textit{H. pylori} positive patients for culturing. Sections of biopsy specimens were embedded in paraffin and stained with Giemsa to detect \textit{H. pylori} and with hematoxylin and eosin in order to evaluate gastric inflammation (mononuclear and polymorphonuclear (PMN) cell infiltration) according to Sydney scoring system (8) on a 4-point scale: no, 0, mild, 1, moderate, 2, and severe, 3. In the group of \textit{H. pylori}-negative subjects, 6 had a non-specific non-steroidal anti-inflammatory drugs (NSAIDs)-related gastritis, while in the remaining the gastric mucosa was normal. The NSAIDs patient median weekly intake in the previous two months was 400 (range 200-700) mg nimesulide per os.

Informed consent was obtained from all patients, and the study protocol was approved by the local ethical committee.

Gastric Biopsy Culture
Antral biopsies of 10 \textit{H. pylori} positive patients were placed on steel grids in an organ culture chamber at 37°C in a 5% CO$_2$/95% O$_2$ with RPMI 1640, 5% fetal bovine serum, 10
mM L-glutamine, 100U/ml penicillin, 100 U/ml streptomycin for 24 hours (all obtained from Invitrogen, Carlsbad, CA) in the presence or absence of a neutralizing IL-12/p70 antibody (5 µg/ml) (R&D Systems, Minneapolis, MN) or control IgG antibody (1 µg/ml) (R&D Systems). At the end, organ culture supernatants were collected and used to measure IFN-γ and IL-4 production by enzyme-linked immunosorbent assay (ELISA), while tissues were used to extract total proteins.

**Extraction of Total Proteins**

Total proteins were extracted from freshly obtained and cultured gastric biopsies using a lysis buffer containing 50 mM HEPES pH 7.6, 150 mM NaCl, 1% Triton X-100, 1 mM Na₃VO₄, 10 mM NaF, 30 mM Na₄P₂O₇, 10% glycerol, 1 mM benzamidine, 1 mM dithiothreitol (DTT), 10 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF) (all obtained from Sigma, Milan, Italy). After incubation for 30 min on ice the membranes were sedimented for 30 min at 14000 rpm at 4°C, and the supernatant was stored at -80°C for later use in Western blot analysis.

**Western blotting**

Total proteins were separated on a 10% sodium dodecyl sulphate-polyacrilamide gel and electrophoretically transferred onto an Immobilon-P membrane (Amersham, Life Sciences Inc., Buckinghamshire, UK). Ponceau S staining was performed to confirm the equal loading and transfer of proteins. The membranes were blocked overnight at 4°C in tris-buffered saline with 0.05% Tween 20 (TBST) (5% non-fat dry milk in 10 mM Tris-HCl, 100 mM NaCl, and 0.1% Tween-20, pH 7.6). This was followed by incubation at room temperature with pSTAT6 and pSTAT4 Abs (all from Invitrogen, Carlsbad, CA), diluted 1/2000 in blocking buffer for 2 hours, and then with horseradish peroxide-conjugated goat anti-rabbit IgG mAb diluted 1/3000 for 1 hour. T-bet Ab was diluted 1/1000 and secondary Ab used was horseradish peroxide-conjugated goat anti-mouse IgG mAb.
diluted 1/3000. The blots of pSTAT4 and pSTAT6 were stripped and reprobed with Abs directed to STAT4 and STAT6, and T-bet were stripped and reprobed with Ab directed to β-actin diluted 1/2000 (all obtained from Santa Cruz Biotechnology). Chemiluminescence luminol reagent (Santa Cruz Biotechnology) was used for detection.

Enzyme Linked Immunosorbent Assay

IL-4, IL-12 and IFN-γ were measured in total protein extracts from freshly gastric biopsies and in 24 hour culture biopsies using lysis buffer [50 mM HEPES pH 7.6, 150 mM NaCl, 1% Triton X-100, 1 mM Na₃VO₄, 10 mM NaF, 30 mM Na₄P₂O₇, 10% glycerol, 1 mM benzamidine, 1 mM dithiothreitol (DTT), 10 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF)]. The total proteins were harvested and cytokine levels were measured using a sensitive ELISA (EuroClone Ltd, Westyorks, UK). One hundred micrograms of total protein extracts were then analyzed for the content of active IL-4, IL-12 and IFN-γ according to the manufacturer’s instruction. Values of active IL-4, IL-12 and IFN-γ were expressed as pg/100 µg of total proteins.

Statistical analysis

Significance of differences was assessed by one-way analysis of variance (ANOVA) and, when the F value was significant, by Tukey’s multiple comparison test. Differences were considered significant if P<0.05. Changes of 30-50% between samples would be considered biologically significant. Thus, to provide 80% power to detect a 30-50% difference with α=0.05, a sample of 6 or 4 patients per group was needed, respectively.
Results

Th1 and Th2 type cytokines are differently produced

The median score for gastric mononuclear cell infiltration was 2.5±0.26, 1.5±0.22, and 0 in the 10 H. pylori-infected, 6, and 4 uninfected patients, P=0.01 and P<0.01, respectively. Subgrouping samples according to gastric mononuclear cell infiltration showed that the higher the inflammation score the higher the level of IFN-γ. Thus, levels of IFN-γ were significantly higher in H. pylori-infected patients who displayed a median inflammation score of 2.5±0.26 (n = 10) compared to uninfected patients having a median inflammation score of 1.5±0.22 (n = 6) (713±10 pg/100µg versus 333±100 pg/100µg, P<0.001) which in turn were higher compared to those in uninfected patients without evidence of gastric mononuclear cell infiltration (n = 4, inflammation score 0) (333±100 pg/100µg versus 15±1 pg/100µg, P<0.001) (Fig. 1, panel A). Otherwise, levels of IL-4 were similar in H. pylori-infected (n = 10) and uninfected (n = 6) patients who displayed gastric mononuclear cell infiltration (median inflammation score 2.5±0.26 and 1.5±0.22; 75±27 pg/100µg versus 64±8 pg/100µg, P=ns, respectively) while were higher in these patients compared with those without evidence of gastric mononuclear cell infiltration (n = 4, inflammation score 0) (75±27 pg/100µg versus 31±1 pg/100µg, P<0.004; and 63±8 pg/100µg versus 31±1 pg/100µg, P<0.001, respectively) (Fig. 1, panel B).

The median score for gastric PMN cell infiltration was significantly higher in H. pylori-infected (n = 10) than uninfected (n = 10) patients (2±0.23 versus 0±13, P<0.001, respectively). Nevertheless, no differences were found in the median PMN score between patients with (n = 6) and without (n = 4) evidence of gastric mononuclear cell infiltration (0±0.21 versus 0, P=ns, respectively).

Increased activation of Th1 transcription factors
Along with the increased release of IFN-γ, the activation of STAT4 (Fig. 2, panel A) and levels of T-bet (Fig. 2, panel B) were found significantly increased in *H. pylori*-infected (n = 10) compared to uninfected patients (n = 10) (0.87±0.22 a.u. versus 0.51±0.18 a.u., p=0.003; and 0.90±0.26 versus 0.46±0.14 a.u., P<0.001, respectively). Furthermore, subgrouping samples according to gastric mononuclear cell infiltration showed that the higher the inflammation score the higher the level of STAT4 and T-bet. Thus, levels of STAT4 and T-bet were significantly higher in *H. pylori*-infected patients displaying a median inflammation score of 2.5±0.26 (n = 10) compared to uninfected patients having a median inflammation score of 1.5±0.22 (n = 6) (0.87±0.22 a.u. versus 0.61±0.07 a.u., P=0.015; and 0.90±0.26 a.u. versus 0.56±0.07 a.u., P=0.004, respectively) which in turn were higher compared to those in uninfected patients without evidence of gastric mononuclear cell infiltration (n = 4, inflammation score 0) (0.64±0.07 a.u. versus 33±0.08 a.u., P<0.001; and 0.56±0.07 a.u. versus 0.31±0.03 a.u., P<0.001, respectively) (Fig. 2, panel A and B). Otherwise, no significant differences in the level of activation of STAT6 (Fig. 3, panel C) were found in *H. pylori*-infected (n = 10) compared to uninfected patients (n = 10) (0.85±0.19 a.u. versus 0.76±0.10 a.u., P=ns), neither when subgrouping according to the score of gastric mononuclear cell infiltration.

**Increased production of IL-12**

Also, an increased release of IL-12 was found in human gastric biopsy cultures from *H. pylori*-infected compared to uninfected patients (Fig. 3). Subgrouping samples according to gastric mononuclear cell infiltration showed that the highest level of IL-12 (22,1±5,3 pg/100µg) was found in *H. pylori*-infected patients (n = 10) who displayed the highest inflammation score (median 2.5±0.26). Otherwise, levels of IL-12 were similar among uninfected patients with (n = 6) and without (n = 4) evidence of gastric mononuclear cell
infiltration (median inflammation score 1.5±0.22 and inflammation score 0; 8.9±1.1 pg/100µg versus 8.43±0.1 pg/100µg, P=ns, respectively).

**Neutralization of IL-12 down-regulates Th1 transcription factors and IFN-γ production**

To examine the role of IL-12 in regulating the production of IFN-γ, gastric biopsies taken from patients with *H. pylori* infection (n = 10) were cultured in the presence or absence of a neutralizing IL-12 antibody or a control antibody. Thus, culturing human gastric biopsies with the neutralizing IL-12 antibody significantly decreased the activation of STAT4 (Fig. 4, panel A) and T-bet (Fig. 4, panel B) (0.91±0.03 a.u. versus 0.66±0.05 a.u., P=0.001; and 0.97±0.01 a.u. versus 0.62±0.01 a.u., P<0.001, respectively). No significant changes were found in the levels of phosphorylated STAT4 and T-bet when using the control antibody. Otherwise, no significant differences in the level of activation of STAT6 (Fig. 4, panel C) were found in gastric biopsies treated with neutralizing IL-12 antibody compared to medium (0.85±0.20 a.u. versus 0.80±0.12 a.u., P=ns) or control antibody. At the same time, presence of neutralizing IL-12 antibody significantly decreased the production of IFN-γ (1197±98 pg/100µg versus 718±173 pg/100µg, P<0.001) (Fig. 5, panel A) while not modifying IL-4 secretion (6.8±0.7 pg/100µg versus 6.5±0.8 pg/100µg, P=ns) (Fig. 5, panel B). No significant changes in the level of IFN-γ and IL-4 were found when using the control antibody.
Discussion

In this study it was confirmed that a Th1 signaling pathway is activated in the human gastric mucosa infected by *H. pylori*. Particularly, the interest focused on the functional role of IL-12. It has been found that the IL-12p70 heterodimer was increased in the gastric mucosa from *H. pylori*-infected patients compared to controls. It is well known that IL-12 binding is rapidly followed by tyrosine phosphorylation of STAT-4 and its translocation to the nucleus. Indeed, levels of active (phosphorylated) STAT-4 were increased in *H. pylori*-infected gastric mucosa compared to uninfected controls. Also, the expression of T-bet and levels of IFN-γ were increased in *H. pylori*-infected samples. In particular, subgrouping of patients showed that up-regulation of Th1 signaling pathway was **associated with high infiltration of the gastric mucosa by mononuclear cells** (i.e., higher in *H. pylori* gastritis than in *H. pylori*-negative gastritis than in normal gastric mucosa). These findings suggest that an important source of mediators of the Th1 response (i.e., cytokines and transcription factors) are mononuclear cells which represent an hallmark of *H. pylori*-related chronic inflammation in the gastric mucosa and a signal that an adaptive immune response has been induced by the bacterium. **Even if the source of IL-12 has not been specifically identified in experiments of this study, it is traditionally considered that macrophages, monocytes and dendritic cells, which are included in our biopsy samples, are the major site of IL-12 production.** On the other hand, specific *H. pylori* virulence factors have been documented to elicit cells of the innate immune system (i.e., PMNs) to produce IL-12 (1). Surprisingly, the *H. pylori*-negative NSAIDs-related gastritis was associated with significantly increased levels of IFN-γ, STAT4 and T-bet, suggesting that NSAIDs-related gastritis is Th1 mediated. However, no elevation in IL-12 levels was found in this subgroup of patients. **The low levels of PMN cell infiltration in NSAIDs-related gastritis, as it has been found in our series, may contribute to the low levels of IL-12 in these patients.** Furthermore, that IL-12 was significantly increased in *H. pylori*
associated gastritis in comparison with *H. pylori*-negative gastritis also in patients with similar scores of gastritis has been already reported (4). Thus, it seems that in this series the Th1-signaling could be driven by mediators other than IL-12. IFN-α and IL-23 are the only cytokines known to induce phosphorylation of STAT4 (6,18). While IFN-α has been shown to be not up-regulated (27) in human gastric mucosa infected by *H. pylori*, no date are available regarding NSAIDs-related gastritis. Therefore, it can be argued that in NSAIDs-related gastritis the activation of STAT4 is predominantly induced by IFN-α and IL-23. Nevertheless, it cannot be excluded that the activation of STAT4 in *H. pylori*-related gastritis is partly induced by IL-23, which shares with IL-12 the p40 subunit and similar biological effects.

No significant differences in the activation of STAT6 were found in *H. pylori*-infected and uninfected human gastric mucosa, irrespective of the intensity of mononuclear cell infiltration. Accordingly, the production of IL-4 was similar in *H. pylori*-infected and uninfected human gastric mucosa who displayed mononuclear cell infiltration at different degrees while it was negligible in uninfected samples without evidence of mononuclear cell infiltration. These data are not surprising in that the same number of IL-4 secreting cells has been already documented in human gastric mucosa with and without *H. pylori* (10). Thus, it appears that IL4 may play a role in modulating the inflammatory process occurring in both *H. pylori* negative and positive gastritis, contributing to down-regulation IL-12 production. Furthermore, data indicate that a Th2 signaling pathway is not induced by *H. pylori*. Nonetheless, to our knowledge this is the first report to date concerning the expression of STAT6 in human gastric mucosa.

Factors which up-regulate Th1 transcription factors in human gastric mucosa infected by *H. pylori* have not been investigated. This study clearly shows, for the first time in ex vivo experiments, that IL-12 plays a role in Th1 signaling pathway in the gastric mucosa of patients with *H. pylori* infection. In fact, the addition of a neutralizing IL-12
antibody to the gastric biopsy cultures resulted in a significant down-regulation of STAT4 and T-bet transcription factors along with a significant decrease of IFN-γ production. Nevertheless, preliminary experiments in our laboratory have shown that anti-IL-12 treatment of *H. pylori* negative gastric samples resulted in a significant down-regulation of STAT4, T-bet and IFN-γ (data not shown). Thus, it appears that also in *H. pylori* negative patients, IL-12 drives, at a lower level, the Th1 response. Mechanisms by which blocking IL-12 results in this effect may be various. It is possible that neutralization of IL-12 inhibits IFN-γ gene activation and/or enhances Th1 cell apoptosis (26,13). Anti-IL-12 antibody could also negatively regulate the activation of STAT4 and expression of T-bet, as is the case in this study. Another possibility may be that an anti-IL-12 treatment could be involved in the increased production of TGF-β1 which eventually leads to the suppression of IFN-γ-secreting cell development (12). Other authors support results of this study showing that IFN-γ is the gene induced by IL-12 in a STAT4 dependent fashion and that defect in Th1 polarization in STAT4 knock-out mice can be restored by adding exogenous IFN-γ to the developing Th1 cells (11). Although STAT4 has been shown to be required for the long-term Th1 differentiation and IFN-γ production (25), cells deficient for STAT4 can differentiate to functional IFN-γ producing Th1 cells (9). Transcription factor T-bet may be involved in the STAT4-independent Th1 differentiation. Indeed, T-bet has been shown to be required for Th1 differentiation and its expression was sufficient to induce IFN-γ production in Th cells (24,23). Induction of T-bet during Th1 commitment leads to remodeling of the IFN-γ locus, induction of IFN-γ production and IL-12Rβ2 expression which is essential for STAT4-mediated IL-12 signaling. Recent evidences suggest that instead of being the primary factor inducing Th1 differentiation (17), STAT4 would rather be involved in enhancing initial IFN-γ production to optimal levels (15).
In conclusion, this study demonstrated that transcription factors STAT4 and T-bet are activated in the human gastric mucosa of *H. pylori*-infected patients. This activation is driven, at least in part, by IL-12 and is associated with increased levels of IFN-γ. Specific targeting of this pathway could be a rational approach for the control of the *H. pylori*-related inflammatory processes in the gastric mucosa. However, the question remains still unanswered as to whether the modulation of the inflammatory response in the human gastric mucosa may be effective in the elimination of the bacterium and, more importantly, in the prevention of *H. pylori*-related sequele.
References


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Figure Legend

Figure 1
IFN-γ (panel A) and IL-4 (panel B) proteins were measured by ELISA in 100µg of total protein extracts obtained for freshly gastric biopsies from H. pylori-infected (n = 10, median inflammation score 2.5±0.26) and uninfected patients with (w) NSAIDs-related (n = 6; median inflammation score 1.5±0.22) and without (w/o, n = 4; inflammation score 0) gastritis. Statistical significance was assessed by ANOVA and Tukey’s multiple-comparison tests.

Figure 2
Activation of Th1 and Th2 transcription factors in human gastric mucosa in H.pylori-infected (n = 10, median inflammation score 2.5±0.26) and uninfected patients with (w) NSAIDS-related (n = 6; median inflammation score 1.5±0.22) and without (w/o, n = 4; inflammation score 0) gastritis. Western blot analysis of phosphorylated (p) STAT4 and STAT4 (panel A), T-bet and β-actin (panel B), and pSTAT6 and STAT6 (panel C) in total proteins extracted from gastric biopsies of 20 patients. A polyacrilamide gel shows in each panel one representative experiment from 10 (lane 1), 6 (lane 2), and 4 (lane 3) patients, respectively. Values are expressed in arbitrary units (a.u.). Statistical significance was assessed by ANOVA and Tukey’s multiple-comparison tests.

Figure 3
IL-12 protein was measured by ELISA in 100µg of total protein extracts obtained for freshly gastric biopsies from H. pylori-infected (n = 10, median inflammation score 2.5±0.26) and uninfected patients with (w) NSAIDS-related (n = 6; median inflammation score 1.5±0.22) and without (w/o, n = 4; inflammation score 0) gastritis. Statistical significance was
assessed by ANOVA and Tukey’s multiple-comparison tests.

Figure 4

Neutralization of endogenous IL-12 results in decreased activation of Th1 transcription factors. Western blot analysis of STAT4 (panel A), T-bet (panel B), and STAT6 (panel C) using total protein extracts from gastric biopsy cultures of 10 *H. pylori* positive patients treated without or with a control IgG (1 µg/ml) or an anti-IL-12 (5 µg/ml) antibody. A polyacrilamide gel shows in each panel one representative experiment from the 10 patients. Values are expressed in arbitrary units (a.u.). Statistical significance was assessed by ANOVA and Tukey’s multiple-comparison tests.

Figure 5

Neutralization of endogenous IL-12 results in decreased IFN-γ but not IL-4 production. IFN-γ (panel A) and IL-4 (panel B) levels were measured by ELISA in 100µg of total protein of supernatant after 24h culture of gastric biopsies from the 10 *H. pylori*-infected patients treated without or with a control IgG (1 µg/ml) or an anti-IL-12 (5 µg/ml) antibody. Statistical significance was assessed by ANOVA and Tukey’s multiple-comparison tests.
FIGURE 1

A

H. pylori+ H. pylori- w gastritis w/o gastritis

P<0.001

P=0.004

P=ns

B

IL-4 (pg/100µg proteins)

IFN-γ (pg/100µg proteins)

H. pylori+ H. pylori-

w gastritis w/o gastritis

P<0.001

P<0.001
FIGURE 2

A

pSTAT-4

STAT-4

H. pylori+

H. pylori-

P=0.003

P<0.001

P=0.015

pSTAT4/STAT4

(a.u.)

B

T-bet

β-actin

H. pylori+

H. pylori-

P<0.001

P<0.001

P<0.001

pSTAT-4

pSTAT-6

STAT-6

H. pylori+

H. pylori-

P=NS

P=NS

P=NS

pSTAT6/STAT6

(a.u.)

w gastritis

w/o gastritis

all

H. pylori+

H. pylori-

P<0.001

P<0.001

P<0.001

H. pylori+

H. pylori-

P=0.004

P=0.004

P=0.004
FIGURE 3

IL-12 (pg/100µg proteins)

Gastrointestinal Inflammation

H. pylori+ w gastritis
H. pylori- w/o gastritis

P<0.001
P=ns
FIGURE 4

A

\[ \text{pSTAT-4/STAT-4} \]

\[ \text{Medium Control Ab Anti IL-12} \]

\[ P=0.001 \]

\[ H. pylori+ \]


B

\[ \text{T-bet/\beta-actin} \]

\[ \text{Medium Control Ab Anti IL-12} \]

\[ P<0.001 \]

\[ H. pylori+ \]


C

\[ \text{pSTAT-6/STAT-6} \]

\[ \text{Medium Control Ab Anti IL-12} \]

\[ P=\text{ns} \]

\[ H. pylori+ \]
FIGURE 5

A

B

H. pylori+

H. pylori+

IFN-γ (pg/100 µg proteins of supernatant)

IL-4 (pg/100 µg proteins of supernatant)

P<0.001

P=ns

Medium

Control Ab

Anti IL-12

P<0.001

P=ns

Medium

Control Ab

Anti IL-12