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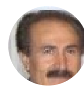

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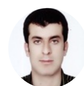

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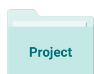

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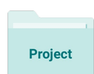

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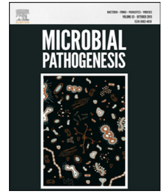

Virulence factors of *Helicobacter pylori vacA* increase markedly gastric mucosal TGF-β1 mRNA expression in gastritis patients

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ABSTRACT

Objective: *Helicobacter pylori* (*H. pylori*) infection is the main cause of gastric inflammation. Regulatory T cells (Treg cells) suppress the activation and proliferation of antigen-specific T cells and mediate immunologic tolerance. TGF-β1 was shown to be secreted in a subset of Treg cells known as 'Th3 cells'. These cells have not been sufficiently studied in context to *H. pylori*-induced inflammation in human gastric mucosa. In this study we therefore, aimed to investigate the expression of TGF-β1 in the context of *H. pylori* colonization in chronic gastritis, to examine the relationship between it and histopathologic findings and to compare it with virulence factors.

Patients and methods: Total RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients and 38 *H. pylori*-negative patients with gastritis. Mucosal TGF-β1 mRNA expression in *H. pylori*-infected and uninfected gastric biopsies was determined by real-time PCR. Presence of *vacA*, *cagA*, *iceA*, *babA2* and *oipA* virulence factors was evaluated using PCR.

Results: TGF-β1 mRNA expression was significantly increased in biopsies of *H. pylori*-infected patients compared to *H. pylori*-uninfected patients. There was association between virulence factors and TGF-β1 mRNA expression. TGF-β1 mRNA expression in mucosa was significantly higher in patients with vacA s1 and s1m1.

Conclusions: TGF-β1 may play an important role in the inflammatory response and promote the chronic and persistent inflammatory changes in the gastric. This may ultimately influence the outcome of *H. pylori*-associated diseases that arise within the context of gastritis and vacA may suffice to induce expression of TGF-β1 mRNA.

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1. Introduction

Colonization by *H. pylori* is known to cause chronic gastritis and lead to the development of severe gastroduodenal diseases such as peptic ulcers, lymphoma of the mucosa associated lymphoid tissue (MALT) or gastric adenocarcinoma [1]. Activation and migration of these inflammatory cells into the gastric mucosa is related to increased production of pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-1β, IL-18, IL-17, IL-8, IL-6 and various cytokines which are believed to contribute to maintaining the gastric inflammation and causing epithelial cell damage [2–5]. The risk of different clinical expression of *H. pylori*-infection is thought to rely on interactions between the host genetic factors and bacterial factors. For instance, polymorphisms of pro-inflammatory cytokine genes such as IL-8, IL-10, IL-17, IL-1β and TNF-α that enhance inflammatory response of gastric mucosa, have been correlated to an increased risk of gastric cancer and peptic ulcer [6–11]. It has been shown that the mucosal levels of several cytokines are significantly higher in *H. pylori*-infected with virulence factors groups in comparison to *H. pylori*-infected without virulence factors groups [12–14]. Even though mucosal *H. pylori* colonization induces vigorous immune responses involving both the innate and adaptive immune system, the infection is not removed, and a state of chronic active

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gastritis persists for life if untreated [15]. During *H. pylori* infection, T cells are generally hyporesponsive and infected gastric tissues have also shown the presence of transforming growth factor β (TGF-β), which has a suppressive effect on T cells [16–18]. Recent studies have demonstrated that $CD4^+$ $CD25^+$ regulatory T cells (Tregs) suppress the immune response against *H. pylori* [19–21]. Mouse model studies have shown that *in vivo* depletion of Tregs in infected mice leads to increased gastric inflammation and reduced colonization of *H. pylori* [20]. The dysfunction or depletion of Treg cells is associated with autoimmune diseases, allergy and inflammatory bowel disease [22]. Today, it is established that regulatory T cells include two distinct subsets, the naturally occurring FOXP3-expressing $CD4^+CD25^{high}$ Treg cells, Tr1 cells secreting interleukin IL-10, and Th3 cells characterized by transforming growth factor (TGF-β1) secretion [23]. Among the $FOXP3^+CD4^+CD25^{high}$ Treg cells, two distinct subsets can be distinguished: (a) thymus derived naturally occurring $FOXP3^+CD4^+CD25^{high}$ Treg cells and (b) peripherally induced $FOXP3^+CD4^+CD25^{high}$ Treg cells. For the differentiation of the latter subset, TGF-β1 has been reported to play an important regulatory role by induction of FOXP3 gene expression in naive $CD4^+$ T cells at the site of inflammation [24]. In animal models, the functional role of Treg cells in the context of *H. pylori*-infection has been investigated, recently. During interaction with *H. pylori*, FOXP3 expressing Treg cells suppressed the immune response toward the bacterium [25]. Gastric Treg cells were able to actively suppress *H. pylori*-induced T-cell proliferation and INF-γ production [19]. Moreover, *H. pylori*-infection is an established risk factor for gastric cancer. In fact, the population of Tregs in tumor-infiltrating lymphocytes is significantly larger than in normal tissue in several malignancies, including gastric cancer [19,26–29]. These findings suggest that Tregs contribute to the persistence of *H. pylori*-infection, which may be closely related to gastric carcinogenesis. Regulatory T cells are thought to be a functionally unique subset of $CD4^+$ T cell populations and function to maintain immune homeostasis [30,31]. The aim of this study was to investigate the expression of TGF-β1 in the context of *H. pylori* colonization in chronic gastritis, to examine the relationship between it and histopathologic findings and to compare it with virulence factors.

2. Materials and methods

2.1. Patients and sampling

In this retrospective study a total of 86 subjects, undergoing endoscopy at Hajar University Hospital, were included (Table 1). The study was approved by the human research ethics committee at Shahrekord University and informed consent was obtained from each volunteer before participation. The groups were characterized as follows: the *H. pylori*–positive group ($n = 48$: 16 males, 32 females; mean age: 42.4 ± 14.82 years); the *H. pylori*–negative group ($n = 38$: 18 males, 20 females; mean age: 38.18 ± 16.51 years). *H. pylori*-infection was determined by the rapid urease test, PCR (16srRNA, glmM) and histological examination of biopsies taken from the corpus. Patients were classified as *H. pylori*-infected only if the four tests were positive, respectively. Four biopsies were collected from 86 *H. pylori*-infected, and used for rapid urease test, histological examination, assessment of bacterial virulence factors, detection of *H. pylori* and cytokine RNA analysis.

2.2. Histological examination

Sections of biopsy specimens were embedded in 10% buffered formalin and stained with hematoxylin and eosin to examine gastritis and with giemsa to detect *H. pylori*. The histological severity of gastritis was blindly graded from normal to severe based on the degree of mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) infiltration, and atrophy according to the Updated Sydney system [32] on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes.

2.3. PCR amplification

DNA for polymerase chain reaction (PCR) was extracted using the biospin tissue genomic DNA extraction kit (BioFlux, Japan). Specific primers for PCR amplification of different genes are shown in Table 1. For vacA, cagA, iceA1, iceA2 and babA2 evaluation, the PCR program comprised 35 cycles of denaturation (at 94 °C for 30 s), annealing (at 56 °C for 30 s, extension at 72 °C for 30 s), and one final extension (at 72 °C for 5 min). For oipA, amplification was performed with 35 cycles of denaturation (at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s), and one final extension (at 72 °C for 5 min).

2.4. Quantitative analysis for TGF-β1 mRNA in the gastric mucosa using real-time PCR

Total RNA was isolated from whole gastric biopsy specimens using total RNA extraction biozol (bioflux, Japan). An aliquot containing 0.2 μg of total RNA was used for the reverse transcription reaction, which was conducted using the superscript first-strand cDNA synthesis system (Fermentas, Finland), according to the manufacturer's instructions. The sequences of oligonucleotide primer and probe are shown in Table 2. The quantification of TGF-β1 mRNA levels was performed using a Rotor-Gene 3000 (Corbett, Australia). Real-time PCR reactions were performed in a total volume of 25 μl containing 3 μl of synthesized cDNA solution, 12.5 μl of 2× Rotor-Gene Probe PCR Master Mix (Qiagen, Germany), 500 nM of each primer and 250 nM of the TaqMan probe. Amplification program included a pre-warming step (10 min at 94 °C), denaturation step (94 °C for 15 s) and an annealing/extension step (60 °C for 60 s). The expression levels of cytokine mRNA were expressed as the ratio of cytokine mRNA to β-actin mRNA (cytokine mRNA/β-actin mRNA). Each assay was performed in duplicate and each cytokine assay was performed testing all RNA samples in the same experiment. Relative quantification of cytokine to β-actin (cytokine mRNA/β-actin mRNA) was determined using $2^{-\Delta Ct} = 2^{-(Ct,\ TGF\text{-}\beta1 - Ct,\ \beta\text{-}actin)}$ method [39].

Table 1
PCR primers for amplification of virulence factors.

Primer designation	Primer sequence	Size of PCR product (bp)	References
vacA m1/m2	vacAmF: 5′-CAATCTGTCCAATCAA GCGAG-3′	567 bp (m1)	[33]
	vacAmR: 5′-GCGTCTAAATAATTCC AAGG-3′	642 bp (m2)	
vacA s1/s2	VA1-F: 5′-ATGGAAATACAACAAA CACAC-3′	259 bp (s1)	[34]
	VA1-R: 5′-CTGCTTGAATGCGCCAAAC-3′	286 bp (s2)	
oipA	oipA-F:CAAGCGCTTAACAGATAGGC-3	430 bp	[35]
	oipA-R:AAGGCGTTTTCTGCTGAAG-3		
iceA1	iceA1F- 5-GTGTTTTTAACCAAAGTATC-3	247 bp	[36]
	iceA1R-5-CTATAGCCASTYTCTTTGCA-3		
iceA2	iceA2F: 5-GTTGGGTATATCACAAT TTAT-3	229 or 334 bp	[36]
	iceA2R: 5-TTRCCCTATTTTCTAGTA GGT-3		
babA2	bab7-F: 5′-CCAAACGAAACAAAAA GCGT-3	271 bp	[37]
	bab7-R: 5′-GCTTGTGTAAAAGCCG TCGT-3		
cagA	cag1: 5-ATGACTAACGAAACTATT GATC-3	232 bp	[38]
	cag2 5-CAGGATTTTTGATCGCTTTATT-3		

Table 2
Primer and probe sequences employed in this study.

Gene	Primer and probe sequence
β-actin	Forward 5-AGCCTCGCCTTTGCCGA-3 Reverse 5-CTGGTGCCTGGGGCG-3 Probe FAM-CCGCCGCCCGTCCACACCCGCC-TAMRA
TGF-β1	Forward 5-CAGCAACAATTCCTGGCGATA-3 Reverse 5-AAGGCGAAAGCCCTCAATTT-3 Probe FAM-CTGCTGGCACCCAGCGACTCG-TAMRA

2.5. Statistical analysis

Data were analyzed using GraphPad Prism 5 Demo (GraphPad Software, San Diego, California, USA). Data are expressed as raw and mean if not stated otherwise. Age was analyzed by unpaired Student's *T*-Test. Normality test was used to determine whether a data set was well-modeled by a normal distribution or not. Cytokine expression was presented as mean and differences between infected and uninfected groups were analyzed using the Student's *T*-Test. Also for gene expression values between *H. pylori*-infected patients and virulence factors, the parametric Student's *T*-Test and for comparison of more than two groups, one-way ANOVA test were used. Categorical data (between virulence factors) are presented as frequencies; comparisons were made using the Chi-square or Fisher exact test if <5 samples were in one group. *P* values <0.05 were considered to be statistically significant.

3. Results

3.1. Genotype

Table 3 gives an overview of the frequency distribution of the vacA s and m alleles and the cagA status. The vacA s1 and m2 alleles as well as cagA were predominant. Mixed vacA s1/s2 genotypes were found in six patients (12.5%) and mixed vacA m1/m2 genotypes were found in four patients (8.3%). The oipA, babA2, iceA1 and iceA2 genes respectively were found in 97.9%, 85.4%, 60.4% and 41.7% of the *H. pylori* positive biopsies (Table 4).

3.2. Correlation of vacA alleles and presence of virulence factors

The cagA gene was detected in 60.4% (29 biopseis) of *H. pylori* positive specimens. The association between vacA alelles and presence of virulence factors is described in Table 5. Of the 29 *H. pylori* positive specimens that were positive for cagA, 26 were associated with the toxin-producing vacA s1 and only 2 cagA-positive were vacA s2 and 16 of them were associated with the toxin-producing vacA s1m2 and only 7 were vacA s1m1 and 2 strains were vacA s2m2.

Table 3
Frequency distribution of the vacA allele variants s and m and the cagA status for the total of study patients analyzed.

Genotype	Total	
	N	%
vacA s-region		
s1	34	70.8
s2	8	16.7
s1 and s2[a]	6	12.5
vacA m-region		
m1	13	27.1
m2	31	64.6
m1 and m2[a]	4	8.3
cagA		
Positive	29	60.4
Negative	19	39.6
Total	48	100%

[a] The mixed genotypes of virulence factors were excluded in quantitative analysis.

Table 4
Frequency of virulence factors in *H. pylori* positive biopsies.

Genotype				Number (%)
oipA	iceA1	iceA2	babA	
+	+	+	+	5 (10.4)
+	+	−	+	20 (41.1)
+	+	−	−	4 (8.3)
+	−	+	+	14 (29.1)
+	−	+	−	1 (2)
+	−	−	+	2 (4.1)
+	−	−	−	1 (2)
−	−	−	−	1 (2)
				48 (100%)

3.3. TGF-β1 expression is enhanced in H. pylori-infected gastric mucosa

TGF-β1 mRNA level was detectable in all samples regardless of whether biopsies were taken from *H. pylori*-infected or uninfected patients. TGF-β1 expression was significantly more in biopsy specimens of *H. pylori*-infected patients compared with uninfected patients ($p = 0.04$) (Fig. 1). If the mean *H. pylori*-positive group is 0.0041 and the mean *H. pylori*-negative group is 0.0004 then the difference in the TGF-β1 mRNA expression in the *H. pylori*-positive compared with the *H. pylori*-negative patients is 0.0041/0.0004 or 8.9-fold.

3.4. Effect of vacA allele variants in H. pylori-infected on the mucosal TGF-β1 mRNA level in gastric mucosa

The results showed that in *H. pylori*-infected patients; mucosal TGF-β1 mRNA level was dependent on the vacA status. The difference in the TGF-β1 mRNA expression in the vacA s1-positive *H. pylori* strains compared with the vacA s2-positive *H. pylori* strains is 0.0045/0.0004 or 10.85-fold. Mucosal TGF-β1 mRNA expression in gastritis patients with vacA m1-positive was not significantly higher than those observed in gastritis patients with vacA m2-positive (0.0081/0.0017 or 4.64-fold). Mucosal TGF-β1 mRNA expression in gastritis patients with vacA s1m1-positive was also significantly higher than those observed in gastritis patients with vacA s1m2-positive (0.0081/0.0026 or 3.11-fold) and vacA s2m2-positive (0.0081/0.0004 or 20.25-fold) (Fig. 2).

3.5. Effect of others virulence factors in H. pylori-infected on the mucosal TGF-β1 mRNA level in gastric mucosa

A mucosal TGF-β1 mRNA level was independent of virulence factors status. If the mean *H. pylori*-positive group is 0.005 and the mean *H. pylori*-negative group is 0.007 then the difference in the TGF-β1 mRNA expression in the cagA-positive *H. pylori* strains compared with the cagA-negative *H. pylori* strains is 0.005/0.0007 or 6.9-fold, the difference in the TGF-β1 mRNA expression in the babA2-positive *H. pylori* strains compared with the babA2-negative *H. pylori* strains is 0.0038/0.0008 or 4.47-fold, the difference in the TGF-β1 mRNA expression in the iceA1-positive *H. pylori* strains compared with the iceA1-negative HP strains is 0.0052/0.0007 or 6.62-fold and the difference in the TGF-β1 mRNA expression in the iceA2-positive *H. pylori* strains compared with the iceA2-negative *H. pylori* strains is 0.0013/0.0048 or 0.27-fold (Fig. 3).

Table 5
Correlation of vacA alleles with the cagA, babA2, iceA1 and iceA2 genotype of the samples studied.

vacA genotype	cagA		babA2		iceA1		iceA2	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
s1m1	7	4	10	1	6	5	4	7
s1m2	16	6	16	6	14	8	8	14
s2m2	2	6	7	1	3	5	4	7
P value	0.06		0.993		0.391		0.524	
s1	26	11	30	7	24	13	12	25
s2	2	6	7	1	3	5	4	4
P value	0.018		0.67		0.157		0.352	
m1	7	4	10	1	6	5	4	7
m2	22	14	29	7	21	15	15	21
P value	0.881		0.429		0.826		0.756	

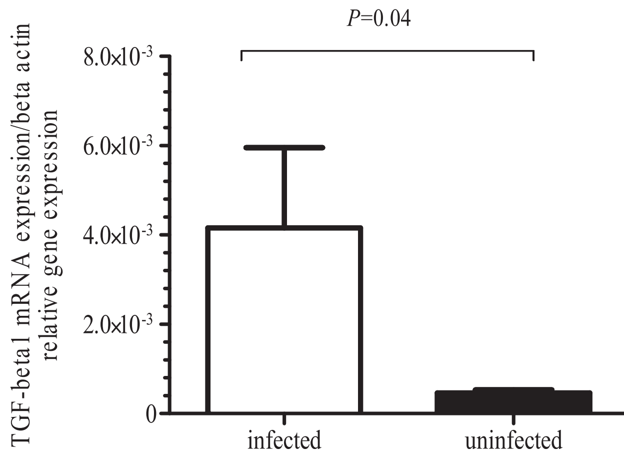

Fig. 1. Relative expression level of TGF-β1 mRNA in gastric mucosa shown by *H. pylori* status. RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients, 38 *H. pylori*-uninfected patients and analyzed for TGF-β1 by real-time PCR. Levels are normalized to β-actin. *P* values <0.05 was considered statistically significant using the Student's *T*-Test.

3.6. Correlation between mucosal TGF-β1 mRNA levels and grade of chronic inflammation

The degree of chronic inflammation was assessed and graded as follows: 31.25% [15] mild, 45.83% [22] moderate and 22.9% [11] severe in the studied population. Fig. 4 shows the relationship between the mucosal TGF-β1 mRNA expression and chronic inflammation (mononuclear cell infiltration) scores. There was a significant correlation between the mucosal TGF-β1 mRNA expression and the chronic inflammation scores ($p = 0.006$).

4. Discussion

Most *H. pylori* infections begin in childhood and persist lifelong in the host without specific treatment. Although *H. pylori* induce a strong gastric inflammation, the pathogens are not cleared, and chronic or persistent inflammation is generally established. The exact mechanisms by which *H. pylori* escapes the bactericidal effects of gastric acid or how it colonizes the gastric mucosa and damages the gastric epithelial cells by *H. pylori* are not well understood. *H. pylori* induced gastric mucosal damage has been

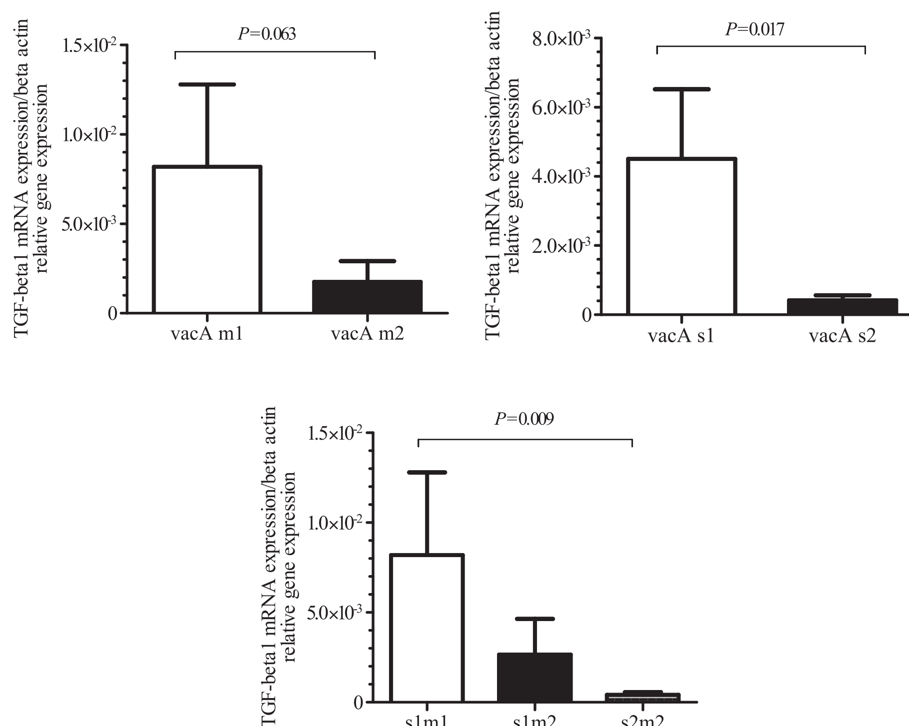

Fig. 2. Relative expression level of TGF-β1 mRNA in *H. pylori*-infected patients shown by vacA allelic variants. RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients (vacA s1 = 34 and s2 = 8 patients) (vacA m1 = 13 and m2 = 31 patients) (vacA s1m1 = 12, s1m2 = 18 and s2m2 = 8 patients).

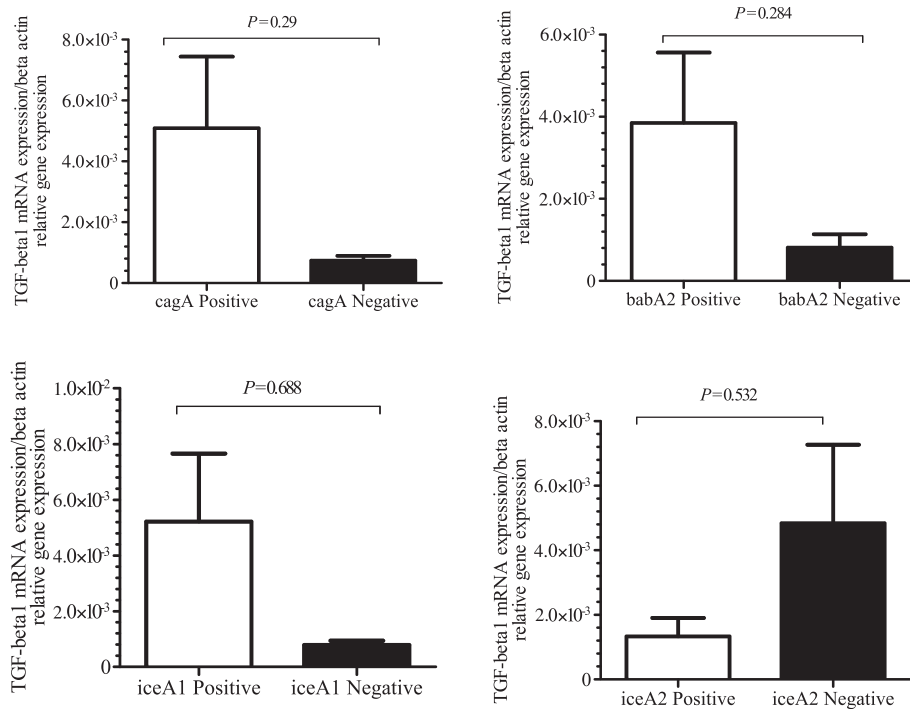

Fig. 3. Mucosal TGF-β1 mRNA expression in gastritis patients and virulence factors. RNA was extracted from gastric biopsies of 48*H. pylori*-infected patients. Levels are normalized to β-actin. *P* values <0.05 was considered statistically significant using the Student's *T*-Test.

explained by an imbalance between host defense mechanisms and bacterial cytotoxic factors. The *H. pylori* vacuolating cytotoxin (vacA) recently has been shown to contribute to persistent infection by inhibiting the proliferation and immune response of T cells [40,41]. Unlike acquired immune system, other investigators [42] have reported that vacA may be a driving factor in efficient nonspecific immune system. The role of vacA in *H. pylori* infected gastric mucosa remains unclear but our results indicate that in *H. pylori*-infected patients; mucosal TGF-β1 mRNA level is dependent on the vacA status. The results indicate that vacA s1 and s1m1 are associated with increased mucosal TGF-β1 mRNA level. Environmental factors, host genetics, considerable genetic diversity and

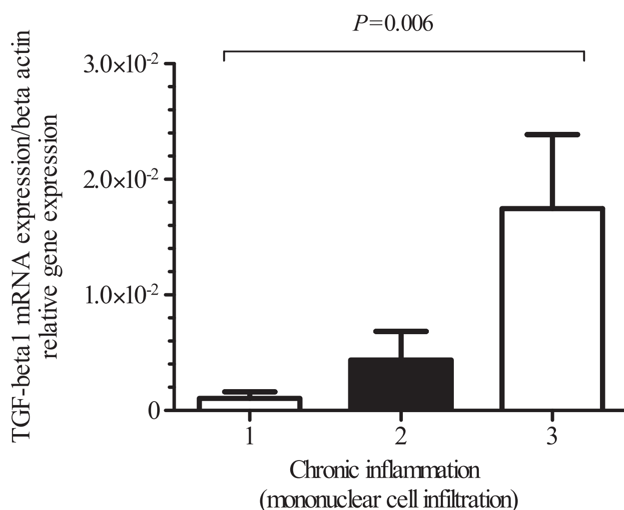

Fig. 4. Relative expression level of TGF-β1 mRNA in *H. pylori*-infected patients shown by grade of gastritis.

pathogenicity between different *H. pylori* strains may contribute to various clinical outcomes [43]. Recent numerous studies have shown that *H. pylori*-induced chronic inflammation could be associated not with a bacterial virulent factor but rather with the host's immunologic tolerance. Treg cells can suppress the proliferation and activation of antigen-specific T cells and they are described as the key regulator of the immune system in the maintenance of immunologic tolerance. Recently, the close correlation between *H. pylori*-infection and immunosuppressive Treg cells in animal and human models has been reported. It has been demonstrated that the depletion of Treg cells in *H. pylori*-infected mice causes an impressive increase in gastric inflammation and a reduction in bacterial colonization [20]. This study has shown that Treg cells suppress Th1-mediated immune response induced by *H. pylori* to contribute to the bacteria's persistent colonization in the gastric mucosa and therefore may play a major role in inducing chronic gastritis. An *in vitro* study found that $CD4^+$ memory T cells from the peripheral blood in *H. pylori*-infected patients were less responsive to *H. pylori* antigens than were cells in *H. pylori*-uninfected, whereas depletion of $FOXP3^+$Treg cells increased the responsiveness of $CD4^+$ memory T cells [44]. This study indicated that $FOXP3^+$Treg cells suppress $CD4^+$ memory T-cell responses to *H. pylori* in infected patients and reduce induction of T cell-mediated mucosal damage and T cell mediated protection against bacterial colonization. Study by Lundgren and colleagues indicated that frequency of $CD4^+CD25^{high}$ Treg cells in gastric mucosa *H. pylori*-infected adults increased, compared to uninfected, using immunohistochemistry [25]. This was the *in vitro* study to examine the suppressive function of Treg cells in gastric mucosa of *H. pylori*-infected adults. Harris and colleagues evaluated the gastric histopathology and Treg cells in *H. pylori*-infected adults and children [45]. In spite of similar *H. pylori* densities, the intensity of gastritis in children was noticeably less than that in adults. Treg numbers and immune suppressive cytokine, IL-10, TGF-β1, were markedly higher in the mucosal of *H. pylori*-infected children compared to infected

adults. This results show that in *H. pylori*-infected children, Treg cell responses downregulate the gastric mucosal inflammation. Associations between the maintenance, development, and induction of Treg cells and TGF-β1 have been studied. Chen and colleagues demonstrated that TGF-β1 induced FOXP3 gene expression in naive $CD4^{+}$T cells at the mucosal of *H. pylori*-infected patients, which was essential for the differentiation of $CD4^{+}CD25^{high}FOXP3^{+}$Treg cells in peripheral blood [46]. These findings introduce $FOXP3^{+}$Treg cells as a major source of TGF-β1 in *H. pylori* gastritis. Sher and Kullberg indicated that Treg cells down regulate Helicobacter hepaticus-induced colonic inflammation in a mouse model of inflammatory bowel disease through the release of TGF-β1 [47]. Both studies showed that the immunosuppressive activity of TGF-β1 was associated with Treg cells. However, the associations between Treg cells and TGF-β1 in *H. pylori*-infection have not yet been comprehensively studied. In the gut of healthy adults, TGF-β1 is an important negative regulator of the Th1 type immune response [48]. TGF-β1 has been reported to be over expressed in the antral of *H. pylori*-infected adults [17]. The results of our study show that *H. pylori*-infection is associated with increased expression of TGF-β1 in children and that $FOXP3^{+}$Treg cells correlate positively with TGF-β1 expression. Our findings also show that the mucosal TGF-β1 mRNA level is increased in the *H. pylori*-infected patients compared with uninfected patients and is correlated positively with vacA, types of disease, and grade of chronic inflammation in patients infected with *H. pylori*. The results indicate that vacA secreted by *H. pylori* may induce the mucosal TGF-β1 mRNA which contributes to persistent infection by inhibiting the proliferation and immune response of T cells. Understanding how *H. pylori* induces Treg cells in patients infected and how Th1 cells appear to finally override the Treg cell influence on *H. pylori*-induced inflammation in adults warrant critical investigation.

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References

[1] Suerbaum S, Michetti P. Helicobacter pylori infection. N Engl J Med 2002 Oct 10;347(15):1175–86 [PubMed PMID: 12374879. Epub 2002/10/11.eng].
[2] Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, et al. Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype. Gastroenterology 1998 Mar;114(3):482–92 [PubMed PMID: 9496938. Epub 1998/03/13.eng].
[3] Yamaoka Y, Yamauchi K, Ota H, Sugiyama A, Ishizone S, Graham DY, et al. Natural history of gastric mucosal cytokine expression in Helicobacter pylori gastritis in Mongolian gerbils. Infect Immun 2005 Apr;73(4):2205–12 [PubMed PMID: 15784564. Pubmed Central PMCID: 1087449. Epub 2005/03/24.eng].
[4] Taylor JM, Ziman ME, Canfield DR, Vajdy M, Solnick JV. Effects of a Th1- versus a Th2-biased immune response in protection against Helicobacter pylori challenge in mice. Microb Pathog 2008 Jan;44(1):20–7 [PubMed PMID: 17683897. Pubmed Central PMCID: 2234601. Epub 2007/08/09.eng].
[5] Bagheri N, Taghikhani A, Rahimian G, Salimzadeh L, Azadegan Dehkordi F, Zandi F, et al. Association between virulence factors of helicobacter pylori and gastric mucosal interleukin-18 mRNA expression in dyspeptic patients. Microb Pathog 2013 Sep 10;65:7–13 [PubMed PMID: 24036181. Epub 2013/09/17.eng].
[6] El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000 Mar 23;404(6776):398–402 [pubMed PMID: 10746728. Epub 2000/04/04.eng].
[7] Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, et al. Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. J Gastroenterol Hepatol 2007 Jan;22(1):51–9 [PubMed PMID: 17201881. Epub 2007/01/05.eng].
[8] Yuzhalin A. The role of interleukin DNA polymorphisms in gastric cancer. Hum Immunol 2011 Nov;72(11):1128–36 [PubMed PMID: 21871937. Epub 2011/08/30.eng].
[9] Wang MY, Chen C, Gao XZ, Li J, Yue J, Ling F, et al. Distribution of Helicobacter pylori virulence markers in patients with gastroduodenal diseases in a region at high risk of gastric cancer. Microb Pathog 2013 Jun–Jul;59–60:13–8 [PubMed PMID: 23583809. Epub 2013/04/16.eng].
[10] Hasegawa S, Nishikawa S, Miura T, Saito Y, Madarame H, Sekikawa K, et al. Tumor necrosis factor-alpha is required for gastritis induced by Helicobacter felis infection in mice. Microb Pathog 2004 Sep;37(3):119–24 [PubMed PMID: 15351034. Epub 2004/09/08.eng].
[11] Hallinger DR, Romero-Gallo J, Peek Jr RM, Forsyth MH. Polymorphisms of the acid sensing histidine kinase gene arsS in Helicobacter pylori populations from anatomically distinct gastric sites. Microb Pathog 2012 Nov–Dec;53(5–6):227–33 [PubMed PMID: 22940419. Pubmed Central PMCID: 3479636. Epub 2012/09/04.eng].
[12] Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Induction of various cytokines and development of severe mucosal inflammation by cagA gene positive Helicobacter pylori strains. Gut 1997;41(4):442–51.
[13] Ando T, Perez-Perez GI, Kusugami K, Ohsuga M, Bloch KC, Blaser MJ. Anti-CagA immunoglobulin G responses correlate with interleukin-8 induction in human gastric mucosal biopsy culture. Clin Diagn Lab Immunol 2000 Sep;7(5):803–9 [PubMed PMID: 10973458. Pubmed Central PMCID: 95959. Epub 2000/09/06.eng].
[14] Bagheri N, Rahimian G, Salimzadeh L, Azadegan F, Rafieian-Kopaei M, Taghikhani A, et al. Association of the virulence factors of Helicobacter pylori and gastric mucosal interleukin-17/23 mRNA expression in dyspeptic patients. EXCLI J 2013;12:5–14.
[15] Ernst PB, Gold BD. The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol 2000;54:615–40 [PubMed PMID: 11018139. Epub 2000/10/06.eng].
[16] Knipp U, Birkholz S, Kaup W, Mahnke K, Opferkuch W. Suppression of human mononuclear cell response by Helicobacter pylori: effects on isolated monocytes and lymphocytes. FEMS Immunol Med Microbiol 1994 Feb;8(2):157–66 [PubMed PMID: 7909699. Epub 1994/02/01.eng].
[17] Li Z, Li J. Local expressions of TGF-beta1, TGF-beta1RI, CTGF, and Smad-7 in Helicobacter pylori-associated gastritis. Scand J Gastroenterol 2006 Sep;41(9):1007–12 [PubMed PMID: 16938712. Epub 2006/08/30.eng].
[18] Lindholm C, Quiding-Jarbrink M, Lonroth H, Hamlet A, Svennerholm AM. Local cytokine response in Helicobacter pylori-infected subjects. Infect Immun 1998 Dec;66(12):5964–71 [PubMed PMID: 9826379. Pubmed Central PMCID: 108755. Epub 1998/11/24.eng].
[19] Enarsson K, Lundgren A, Kindlund B, Hermansson M, Roncador G, Banham AH, et al. Function and recruitment of mucosal regulatory T cells in human chronic Helicobacter pylori infection and gastric adenocarcinoma. Clin Immunol 2006 Dec;121(3):358–68 [PubMed PMID: 16934529. Epub 2006/08/29.eng].
[20] Rad R, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, et al. CD25+/Foxp3+ T cells regulate gastric inflammation and Helicobacter pylori colonization in vivo. Gastroenterology 2006 Aug;131(2):525–37 [PubMed PMID: 16890606. Epub 2006/08/08.eng].
[21] Raghavan S, Suri-Payer E, Holmgren J. Antigen-specific in vitro suppression of murine Helicobacter pylori-reactive immunopathological T cells by CD4CD25 regulatory T cells. Scand J Immunol 2004 Jul–Aug;60(1–2):82–8 [PubMed PMID: 15238076. Epub 2004/07/09.eng].
[22] Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005 Apr;6(4):345–52 [PubMed PMID: 15785760. Epub 2005/03/24.eng].
[23] Taams LS, Palmer DB, Akbar AN, Robinson DS, Brown Z, Hawrylowicz CM. Regulatory T cells in human disease and their potential for therapeutic manipulation. Immunology 2006 May;118(1):1–9 [PubMed PMID: 16630018. Pubmed Central PMCID: 1782265. Epub 2006/04/25.eng].
[24] Fu S, Zhang N, Yopp AC, Chen D, Mao M, Zhang H, et al. TGF-beta induces Foxp3 + T-regulatory cells from CD4 + CD25-precursors. Am J Transpl 2004 Oct;4(10):1614–27 [PubMed PMID: 15367216. Epub 2004/09/16.eng].
[25] Lundgren A, Stromberg E, Sjoling A, Lindholm C, Enarsson K, Edebo A, et al. Mucosal FOXP3-expressing CD4+ CD25high regulatory T cells in Helicobacter pylori-infected patients. Infect Immun 2005 Jan;73(1):523–31 [PubMed PMID: 15618192. Pubmed Central PMCID: 538965. Epub 2004/12/25.eng].
[26] Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clin Cancer Res 2006 Sep 15;12(18):5423–34 [PubMed PMID: 17000676. Epub 2006/09/27.eng].
[27] Mizukami Y, Kono K, Kawaguchi Y, Akaike H, Kamimura K, Sugai H, et al. Localisation pattern of Foxp3+ regulatory T cells is associated with clinical behaviour in gastric cancer. Br J Cancer 2008 Jan 15;98(1):148–53 [PubMed PMID: 18087278. Pubmed Central PMCID: 2359685. Epub 2007/12/19.eng].
[28] Perrone G, Ruffini PA, Catalano V, Spino C, Santini D, Muretto P, et al. Intratumoural FOXP3-positive regulatory T cells are associated with adverse prognosis in radically resected gastric cancer. Eur J Cancer 2008 Sep;44(13):1875–82 [PubMed PMID: 18617393. Epub 2008/07/12.eng].
[29] Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. Cancer Res 2005 Mar 15;65(6):2457–64 [PubMed PMID: 15781662. Epub 2005/03/23.eng].
[30] Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4(+)CD25(+) T cells with

regulatory properties isolated from peripheral blood. J Exp Med 2001 Jun 4;193(11):1285–94 [PubMed PMID: 11390435. Pubmed Central PMCID: 2193380. Epub 2001/06/08.eng].

[31] Ng WF, Duggan PJ, Ponchel F, Matarese G, Lombardi G, Edwards AD, et al. Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. Blood 2001 Nov 1;98(9):2736–44 [PubMed PMID: 11675346. Epub 2001/10/25.eng].

[32] Manxhuka-Kerliu S, Telaku S, Devolli-Disha E, Ahmetaj H, Sahatciu-Meka V, Kerliu A, et al. Helicobacter pylori gastritis updated Sydney classification applied in our material. Prilozi 2009 Jul;30(1):45–60 [PubMed PMID: 19736530. Epub 2009/09/09.eng].

[33] Van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, et al. Geographic distribution of vacA allelic types of Helicobacter pylori. Gastroenterology 1999 Apr;116(4):823–30 [PubMed PMID: 10092304. Epub 1999/03/26.eng].

[34] Atherton JC, Peek Jr RM, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of Helicobacter pylori. Gastroenterology 1997 Jan;112(1):92–9 [PubMed PMID: 8978347. Epub 1997/01/01.eng].

[35] Atherton JC, Cover TL, Twells RJ, Morales MR, Hawkey CJ, Blaser MJ. Simple and accurate PCR-based system for typing vacuolating cytotoxin alleles of Helicobacter pylori. J Clin Microbiol 1999 Sep;37(9):2979–82 [PubMed PMID: 10449485. Pubmed Central PMCID: 85427. Epub 1999/08/17.eng].

[36] van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, et al. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. Gastroenterology 1998 Jul;115(1):58–66 [PubMed PMID: 9649459. Epub 1998/07/03.eng].

[37] Sheu BS, Sheu SM, Yang HB, Huang AH, Wu JJ. Host gastric Lewis expression determines the bacterial density of Helicobacter pylori in babA2 genopositive infection. Gut 2003 Jul;52(7):927–32 [PubMed PMID: 12801945. Pubmed Central PMCID: 1773709. Epub 2003/06/13.eng].

[38] Lobo Gatti L, Agostinho Jn F, De Labio R, Balbo Piason F, Carlos Da Silva L, Fagundez De Queiroz V, et al. Helicobacter pylori and cagA and vacA gene status in children from Brazil with chronic gastritis. Clin Exp Med 2003 Nov;3(3):166–72 [PubMed PMID: 14648232. Epub 2003/12/04.eng].

[39] Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008;3(6):1101–8 [PubMed PMID: 18546601. Epub 2008/06/13.eng].

[40] Gebert B, Fischer W, Weiss E, Hoffmann R, Haas R. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. Science 2003 Aug 22;301(5636):1099–102 [PubMed PMID: 12934009. Epub 2003/08/23.eng].

[41] Kimura M, Goto S, Ihara Y, Wada A, Yahiro K, Niidome T, et al. Impairment of glutathione metabolism in human gastric epithelial cells treated with vacuolating cytotoxin from Helicobacter pylori. Microb Pathog 2001 Jul;31(1): 29–36 [PubMed PMID: 11427034. Epub 2001/06/28.eng].

[42] Hisatsune J, Nakayama M, Isomoto H, Kurazono H, Mukaida N, Mukhopadhyay AK, et al. Molecular characterization of Helicobacter pylori VacA induction of IL-8 in U937 cells reveals a prominent role for p38MAPK in activating transcription factor-2, cAMP response element binding protein, and NF-kappaB activation. J Immunol 2008 Apr 1;180(7):5017–27 [PubMed PMID: 18354227. Pubmed Central PMCID: 3404739. Epub 2008/03/21.eng].

[43] Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H. Helicobacter pylori endemic and gastric disease. Dig Dis Sci 2005 Nov;50(11):2075–80 [PubMed PMID: 16240218. Epub 2005/10/22.eng].

[44] Lundgren A, Suri-Payer E, Enarsson K, Svennerholm AM, Lundin BS. Helicobacter pylori-specific CD4+ CD25high regulatory T cells suppress memory T-cell responses to H. pylori in infected individuals. Infect Immun 2003 Apr;71(4):1755–62 [PubMed PMID: 12654789. Pubmed Central PMCID: 152046. Epub 2003/03/26.eng].

[45] Harris PR, Wright SW, Serrano C, Riera F, Duarte I, Torres J, et al. Helicobacter pylori gastritis in children is associated with a regulatory T-cell response. Gastroenterology 2008 Feb;134(2):491–9 [PubMed PMID: 18242215. Epub 2008/02/05.eng].

[46] Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 2003 Dec 15;198(12):1875–86 [PubMed PMID: 14676299. Pubmed Central PMCID: 2194145. Epub 2003/12/17.eng].

[47] Sher A, Kullberg M. Effector and regulatory CD4+ T cell function in a murine model of Helicobacter hepaticus-induced colitis. J Pediatr Gastroenterol Nutr 2005 Apr;40(Suppl. 1):S35–6 [PubMed PMID: 15805844. Epub 2005/04/05.eng].

[48] Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006;24:99–146 [PubMed PMID: 16551245. Epub 2006/03/23.eng].

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