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Full Paper

Changes in the tissue elements of the gastric mucosa interacting with different strains of *Helicobacter pylori*, taking into consideration the patient's genotype

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The present study aimed to investigate the peculiarities of adaptation of tissue elements of the gastric mucosa during interaction with *Helicobacter pylori*, as determined by genetic characteristics of the bacterium and the host. Venous blood and biopsy samples of the mucosa of the antrum and body of the stomach from young patients (18 to 25 years old) were examined. The condition of the gastric mucosa was assessed using stained histological preparations. Venous blood was collected from the patients to ascertain the polymorphisms of the IL-lß and IL-IRN genes. The most pronounced changes were observed in the parameters of reparative regeneration of epithelial differentiation during colonization of the gastric mucosa by *H. pylori* strains carrying the CagA(+) and BabA2(+) genes. These included an increase in proliferation and apoptosis rates and alterations in epithelial differentiation markers characterized by elevated production of Shh and MUC5AC, as well as a reduction in the production of the protective mucin MUC6 by isthmus gland cells. The presence of the vacAs1 and vacAs2 genes of *H. pylori* results in a high level of apoptosis in epithelial cells without accelerating proliferation. It was found that after eradication, patients with preserved cellular infiltrates in their gastric mucosa plates were carriers of mainly the IL-1ß*T/IL-1RN*2R haplotypes after 12 months.

Key words: *Helicobacter pylori*, strains, gastric mucosa, cellular renewal, genetic polymorphism

INTRODUCTION

According to modern concepts, *Helicobacter pylori* is considered the primary etiologic factor of chronic inflammatory diseases of the upper gastrointestinal tract [1–3]. Currently, the consensus is that the detection of *H. pylori* in most cases automatically results in the prescription of an anti-*H. pylori* treatment under the current Maastricht V agreement, Kyoto global consensus on *H. pylori*-associated gastritis [4, 5]. A sizeable prevalence of *H. pylori* has been observed in Russia. According to some data, almost 70–80% of the population is infected. Furthermore, the problems of antibiotic resistance and the inefficiency of eradication therapy remain highly relevant [6, 7]. At the same time, when the gastric mucosa is colonized with low-pathogenic strains of *H. pylori* without pronounced clinical and morphological changes, standard eradication schemes cannot always be considered justified [8, 9].

Studies have shown that *H. pylori* regulates cellular renewal processes in epitheliocytes directly through the action of urease and lipopolysaccharide and indirectly through the induction of cytokine secretion by inflammatory infiltrate cells [10]. Cell turnover parameters were positively correlated with the degree of inflammation of the gastric mucosa [11]. *H. pylori* infection has been shown to disrupt cell turnover processes in the gastric mucosa. In particular, *H. pylori* stimulates the apoptosis of glandular epithelial cells of the stomach, causing hyperproliferation in them [12].

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It is known that *H. pylori* is characterized by high genetic polymorphism, which determines its virulence and pathogenicity [13]. The peculiarities of the genetic structure of an infectious agent may significantly affect the nature of changes in the mucosa of the upper gastrointestinal tract. As a result, they may affect the development, course, and prognosis of gastric diseases, as well as the effectiveness of therapy [14–16]. This demonstrates the importance of studying the genetic structure of *H. pylori* in helicobacter-associated diseases of the stomach and duodenum.

The relationship between *H. pylori* and the patient's body may differ between cases. In some cases, *H. pylori* initiates the development of a chronic inflammatory process in the stomach and duodenum's mucous membrane; in others, it acts as a commensal [17]. Currently, there are data indicating that not only bacterial properties but also genetic peculiarities of the infected person (host) determine the development of reactive changes in the stomach mucous membrane [18]. Functional polymorphisms of genes responsible for recognizing pathogens and their actions during the initial stage of inflammation influence the character of protective reactions and the physiology of the gastric mucosa [19–21].

Thus, under modern concepts, the heterogeneity of the inflammatory response to *Helicobacter* infection is due to the genetic characteristics of the individual [22]. However, the understanding of the influence of cytokine gene polymorphism on the cell renewal of the gastric mucosal epithelium in the event of *Helicobacter* infection remains incomplete. The present study aimed to establish the peculiarities of the adaptation of tissue elements of the gastric mucosa during interaction with *H. pylori*, as determined by genetic characteristics of the bacterium and host.

MATERIALS AND METHODS

Study design

Biopsy specimens of the mucous membrane of the antrum and body of the stomach, obtained during fibrogastroduodenoscopy of young patients (18–25 years old; mean age, 21 ± 2.2 years old) infected with *H. pylori* bacteria, were studied. The study was a cohort, prospective, controlled, comparative study in nature, with elements of a retrospective analysis.

The criteria for inclusion in the study were as follows: a) presence of clinical dyspepsia syndrome (epigastric pain, nausea, heartburn, feeling of fullness of the stomach, vomiting) and b) informed consent of the patients for the study. The exclusion criteria were as follows: a) receipt of eradication therapy before the experiment, b) use of antisecretory or nonsteroidal anti-inflammatory drugs in the preceding three months, and c) concomitant allergic or immunopathological diseases that could impact the effectiveness of the infectious-host interaction.

All patients in the study were residents of Moscow, not related, and ethnically Russian. The following groups were formed based on the purpose and objectives of the study:

(a) Group I (22 patients), comprising 44 biopsy samples from the antral mucosa and stomach body colonized by *H. pylori* with superficial mucous lesions

(b) Group II (19 patients), comprising 38 biopsy samples of the antral mucosa and body of the stomach colonized by *H. pylori*, with erosive and ulcerative mucous lesions

(c) Group III (comparison, 16 patients), comprised of 32 biopsy samples of the anterior mucosa and gastric body without colonization with *H. pylori* (unchanged mucous membranes or minimal alterations)

Venous blood samples were taken from all study groups for genetic study. Additionally, another group, Group IV (control), was formed using venous blood samples from 32 individuals who exhibited no indications of *H. pylori* infection and dyspepsia syndrome. These samples were employed for analysis of the outcomes of selective genetic research. There were no statistically significant differences in age or gender among all study groups. The average duration of clinical dyspepsia syndrome was 4.2 ± 2.6 years.

All patients diagnosed with *H. pylori*-positive gastritis were administered anti-*Helicobacter* therapy according to the guidelines outlined in Maastricht Consensus II [23]. Dynamic patient observation was conducted at three key time points: before *H. pylori* eradication, at 2 months post anti-*Helicobacter* therapy (for eradication control), and at 12 months (to monitor for reinfection) following treatment. At the initial checkpoint, immunohistochemical and morphometric analyses of antral mucosa and stomach body biopsies were carried out, along with genotyping of the *H. pylori* strain. At the second and third checkpoints, immunohistochemical and morphometric evaluations of antral mucosa and stomach body biopsies were conducted.

Cases exhibiting ineffective infection eradication at the 2-month mark and reinfection at the 12-month interval were excluded from the study. Consequently, at the second observation point (1–2 months post-eradication), 36 biopsies from Group I (18 patients) and 34 biopsies from Group II (17 patients) were examined. At the third observation point (12 months post-eradication), 32 (16 patients) and 30 (15 patients) biopsies were assessed in Groups I and II, respectively. A genetic investigation of IL-1β and IL-1RN gene polymorphism was conducted among patients.

Immunohistochemical methods

Diagnostics of *Helicobacter* infection were performed according to the recommendations of the Maastricht Consensus Methods for histopathological examination of mucous biopsy samples, and the Helpil urease rapid test and polymerase chain reaction (PCR) were used. All patients underwent gastroduodenoscopy with targeted biopsy of the mucosal lining of the stomach's body and the anterior portion of the stomach during the diagnostic procedures at the 2-month and 12-month intervals following anti-*Helicobacter* therapy. Two fragments of the mucosa were collected in the anterior part of the stomach for histological examination and detection of *H. pylori* by PCR, and one fragment of the mucous membrane from the stomach body was collected for histological examination. Biopsies of gastric mucous membranes were fixed in a 10% neutral buffered formalin solution (pH 7.2–7.4) within 12–24 hr and incorporated into paraffin according to the standard procedure [5]. The condition of the gastric mucous membrane was assessed using histological preparations stained with hematoxylin and eosin. Morphological changes were evaluated with a visual analogue scale developed based on the Sydney classification system of chronic gastritis [24], using the following grades: none, low, moderate, and high degree of symptom manifestation.

The composition of mucins in the gastric mucosa was determined by staining techniques with a combination of Alcian

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Blue (pH 2.5) and a periodic acid-Schiff (PAS) reaction to detect sialomucins and neutral mucins [25]. An iron diamine reaction in combination with Alcian Blue (pH 2.5) was used to detect sulfamucins. Van Gieson's picrofuchsine reaction was applied to detect the connective tissue zones. An immunohistochemical study was conducted on paraffin sections using the streptavidin-biotin method (LSAB 2 System HRP, DAKO, Denmark) [25]. A list of antibodies utilized in the study is presented in Table 1.

Morphometric methods

Linear morphometry of the mucous membrane thickness, gastric pit depth, cover epithelium height, and pit epithelium of biopsy samples (in μm) was conducted using an ocular micrometer. The cellular composition and density of infiltrate in 1 mm^2 of gastric mucosa and volume fractions in percentage of the mucosal epithelium, glands, interepithelial lymphocytes, stroma, and cellular infiltrate of the proper mucosa plate and lymphoid nodules were analyzed using Avtandilov's grid [26].

Following the immunohistochemical reaction, the proliferation (nuclear label Ki67) and apoptosis (perinuclear or cytoplasmic label CPP32) indices, expression of apoptosis regulatory proteins (cytoplasmic Bcl-2 labelling), and production of intestinal transcription factor (CDX-2 labelling) were determined in tissue sections at 400× magnification under a microscope as a percentage of positively stained nuclei in gastric mucosal epithelial cells within five randomly selected fields of view (≥500 cells) [8]. Given the observed mosaic distribution of the labelled cells within the gastric mucosa, additional assessments of labelling indices were separately conducted in three functional zones, the superficial and pit epithelium (I), isthmus zone (II), and base (III), as well as the middle and lower third of the glands.

The production of Shh gastric differentiation factor was recorded as an index of cytoplasmic staining intensity, as follows: 1, weak; 2, moderate; and 3, severe [8]. The percentage of positively colored cells among the total number of epithelial cells (>500 cells) was ranked according to five gradations (0, up to 5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; 4, 75% and more) of positively stained cells. An estimated Shh production index was calculated by multiplying the staining intensity index (1–3) by the semiquantitative ranking (0–4) [8]. A semiquantitative scoring system was employed to assess the expression of mucins (MUC5AC, MUC6, MUC2) in the gastric mucosa. This system utilized a scale for evaluating the extent of staining as follows: 0 (absent), no positive reaction in cells; 1 (weak), staining in up to 30% of cells; 2 (moderate), staining in 31–60% of cells; 3 (strong), staining in 60% or more of the cells.

PCR method

H. pylori genotyping was performed by PCR (Helicopol-II test system, Litech Scientific Production Company, Russia) with antral gastric biopsy samples. The presence of cagA and babA2 genes, as well as polymorphisms of vacA genes by the signal and middle sites (s1/s2 and m1/m2 subtypes, respectively) and iceA (iceA1/iceA2 subtypes), was established [20]. To determine IL-lß and IL-IRN gene polymorphisms, venous blood (4–5 mL) was collected with an anticoagulant, followed by obtaining a leukocyte suspension. Then, the DNA was isolated by phenol-chloroform extraction with ethanol precipitation using oligonucleotide primers. Polymorphism of the IL-IRN gene was investigated with primers flanking the polymorphous region within the second intron, which contains a variable number of tandem repeats (VNTR) of 86 bp [21]. As a result of amplification, 438 bp, 524 bp, 610 bp, 696 bp, and 782 bp DNA fragments with 2, 3, 4, 5, or 6 tandem repeats were identified, respectively. PCR with additional restriction of amplicons by endonuclease restriction was used to identify polymorphic variants of the IL-lß gene: TaqI for genotyping the polymorphic T locus C 3953 and Ama871 for genotyping the polymorphic T locus C -511 [21].

To detect *H. pylori*-positive gastritis, anti-*Helicobacter* therapy based on the provisions approved by the Maastricht Accords on diagnosing and treating *H. pylori* infection was administered.

Patients were followed for 1–2 months (to control eradication of *H. pylori*) and 12 months (to control reinfection) after treatment. Cases of ineffective infection eradication at 1–2 months and reinfection at 12 months were excluded from the study.

Statistical analysis

The arithmetic mean and standard deviation were used as descriptive statistics for principal characteristic data with a normal distribution. Also, Student's parametric criterion was used for data with a normal distribution. The Bonferroni correction coefficient was used to benchmark the variables in the three groups. If the distribution was not normal, non-parametric criteria were applied, with categorical data analyzed by chi-square test (χ^2) with Yates continuity correction and numerical data analyzed by Mann–Whitney U test. For multiple comparisons (three groups), the

Table 1. List of antibodies utilized in the study

Antibody name	Clone	Marker value	Working dilution	Manufacturer
Ki67	MIB-1	Proliferation marker	Ready to use	«DAKO», Denmark
CPP32	JHM62	Apoptosis marker	1:25	«Novocastra», United Kingdom
Bpl -2	124	Suppression of apoptosis marker	Ready to use	«DAKO», Denmark
CD4	CD4-1F6	Membrane marker for helper T-Lymphocyte subpopulation	Ready to use	«Novocastra», United Kingdom
CD8	CD8-295	Membrane marker for cytotoxic T-Lymphocyte subpopulation	Ready to use	«Novocastra», United Kingdom
CD20	L26	Membrane marker for B-Lymphocytes	Ready to use	«DAKO», Denmark
MUC5AC	CLH2	Neutral mucin of surface regions of gastric mucosal epithelium. Antibody to apoprotein	1:100	«Novocastra», United Kingdom
MUC6	CLH5	Acidic mucin of deep gastric gland regions. Antibody to apoprotein	1:100	«Novocastra», United Kingdom
MUC2	Ccp58	Acidic mucin of intestinal type. Antibody to apoprotein	1:200	«Novocastra», United Kingdom
CDX-2	CDX2-88	Factor enabling intestinal epithelium differentiation	1:150	«Cell Mark», USA
Shh	Polyclonal	Factor enabling gastric epithelium differentiation	1:100	«Spring», USA

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non-parametric Dunn criterion was employed. Spearman's degree coefficient (p) was used for correlation analysis. The critical level of significance for statistical testing hypotheses was p=0.05. The strength of relationships was evaluated based on odds ratio (OR) values [27].

RESULTS

Characteristics of the gastric mucosa depending on strain-specific features of H. pylori

The cagA, babA2, and iceA (predominantly genotype IceAl) strains were significantly more frequently identified in biopsy specimens from patients with erosive-ulcerative lesions of the gastric mucosa (Group II) compared with the group of patients with superficial lesions (Group I): 53%, 26%, and 76% vs. 32% (χ^2=4.235; p=0.026), 11% (χ^2=4.362; p=0.018), and 57% (χ^2=5.158; p=0.008) of cases, respectively (Table 2). In this context, the babA2-positive genotype of *H. pylori* was consistently linked to the cagA genotype in 100% of cases within both of these groups. It is noteworthy that in the studied biopsies of these two groups, less cytotoxic strains of *H. pylori*—vacAs2/mL and vacAs2/m2—were more frequently identified (Table 3).

In addition, when the intrinsic lamina of the gastric antral mucosa was infected with cagA(+), babA2(+), iceAl(+), vacAsl/ml, and vacAsI/m2 strains of *H. pylori*, a semiquantitative assessment identified either a pronounced or moderate degree of inflammatory infiltrate consisting of lymphocytes, macrophages, and neutrophilic leukocytes. In the case of infection with cagA(−), babA2 (−), iceA2(+), vacAs2/mL, and vacAs2/m2 strains, the degree was stated as moderate or weak.

The results of the immunohistochemical study of cellular renewal of the gastric mucous membrane according to the specific characteristics of the *H. pylori* strain demonstrated that the greatest changes in the index of cellular renewal were observed when the gastric mucous membrane was infected with cagA(+) and babA2(+) strains. A significant increase in epithelial cell apoptosis index was also observed in the gastric mucosa when infected with vacAsl(+) or vacAs2(+) strains. However, this was not accompanied by a parallel increase in the proliferation of these cells (Table 2).

Analysis of gastric mucosal epithelial renewal during infection with multiple pathogenic strains of H. pylori

The results identified 22 combinations of vacA, cagA, babA2, and iceA genotypes of *H. pylori.* In the studied biopsy specimens of Groups I and II, *H. pylori* strains with the vacAs2/m2, cagA(−), and iceA2 and vacAs2/m2, cagA(−), and iceAl genotypes respectively, were most common (Table 3).

Statistically significant differences in the percent proliferation indices were observed in biopsy specimens from Group I (23.8 ± 4.8 and 13.4 ± 4.2, respectively, p=0.003) and Group II (29.4 ± 6.5 and 14.8 ± 4.3, respectively, p=0.012) infected with a combination of the cagA(+)vacAs1(+) and cagA(+)vacAsl(−) strains. The percentage apoptosis index was approximately the same regardless of the cagA(+)vacAsl(+) or cagA(−)vacAs 1 (+) strains of *H. pylori* in both Group I (14.5 ± 3.8 and 14.1 ± 4.4, respectively) and Group II (19.6 ± 4.9 and 18.4 ± 4.3, respectively) patients.

Analysis of the cellular renewal of the gastric mucosa during infection by several pathogenic *H. pylori* strains revealed patterns

Table 2. Average proliferation index (IP, nuclear label Ki67) and apoptosis index (IA, perinuclear or cytoplasmic label CPP32) of gastric mucosa epithelial cells during infection with different *Helicobacter pylori* strains

Indexes	Group I (n=44)		Group II (n=38)	
	CagA(+), n=14	CagA(−), n=30	CagA(+), n=20	CagA(−), n=18
IP	28.2 ± 8.2	13.3 ± 6.8*	28.9 ± 6.8	13.7 ± 4.8*
IA	11.7 ± 3.8	5.8 ± 2.7*	25.2 ± 6.3	10.3 ± 4.7*
	BabA2(+), n=5	BabA2(−), n=39	BabA2(+), n=10	BabA2(−), n=28
IP	24.3 ± 6.8	11.5 ± 4.3*	25.8 ± 8.2	16.8 ± 6.5*
IA	12.8 ± 4.1	9.7 ± 3.7	24.7 ± 6.3	14.6 ± 6.2*
	VacAs1/mL(+), n=7	VacAs1/mL(−), n=37	VacAs1/mL(+), n=7	VacAs1/mL(−), n=31
IP	23.2 ± 4.3	14.1 ± 3.6	24.3 ± 6.5	19.2 ± 4.1
IA	16.8 ± 3.2*	9.3 ± 3.4	14.7 ± 3.9*	8.1 ± 3.3
	VacAs1/m2(+), n=12	VacAs1/m2(−), n=32	VacAs1/m2(+), n=12	VacAs1/m2(+), n=26
IP	11.7 ± 3.2	10.2 ± 6.7	19.8 ± 5.6	18.2 ± 4.5
IA	11.8 ± 3.8*	6.9 ± 2.1	13.8 ± 2.8*	7.3 ± 4.5
	VacAs2/mL(+), n=15	VacAs2/mL(−), n=29	VacAs2/mL(+), n=10	VacAs2/mL(+), n=28
IP	18.2 ± 4.3	14.4 ± 4.2	20.2 ± 5.2	15.9 ± 4.4
IA	11.9 ± 4.6*	8.4 ± 3.4	18.1 ± 2.9	15.7 ± 2.8
	VacAs2/m2(+), n=22	VacAs2/m2(−), n=22	VacAs2/m2(+), n=23	VacAs2/m2(+), n=15
IP	17.7 ± 4.3	18.7 ± 5.3	24.2 ± 5.4	25.2 ± 5.6
IA	10.2 ± 3.8*	7.2 ± 5.2	15.6 ± 4.6	14.3 ± 3.8
	IceA1(+), n=16	IceA1(−), n=12	IceA1(+), n=19	IceA1(−), n=6
IP	22.5 ± 7.1	18.1 ± 4.2	27.6 ± 6.9	25.8 ± 6.8
IA	9.6 ± 5.2	10.7 ± 4.2	22.4 ± 6.2	20.2 ± 4.3
	IceA2(+) n=7	IceA2(−), n=9	IceA2(+) n=8	IceA2(−), n=5
IP	19.6 ± 6.2	21.2 ± 4.2	28.2 ± 7.4	21.2 ± 6.5
IA	9.8 ± 6.5	12.1 ± 2.5	19.7 ± 5.6	18.8 ± 4.6

*Differences are significant between positive and negative *Helicobacter pylori* strains in the studied groups, p<0.05.

in the distribution of immunohistochemically labelled cells in the epithelial differon.

In biopsy specimens of the antral mucosa and body of the stomach with an unchanged mucosa (Group III), expression of nuclear antigen Ki67 (proliferation marker) was determined mainly in the area of the isthmus gland. The bacterium in the epithelial differon facilitated widening of the Ki67-stained area towards the fossa and into the deep parts of the glands (Groups I and II), and in some cases, it facilitated widening to the tops of the rolls (Groups I and II). The number of labelled CPP32 cells in biopsy specimens of the antrum at mucosa colonization by *H. pylori* (Groups I and II) in comparison with biopsy specimens with an unchanged mucosa (Group III) was increased in the area of the papillary epithelium and deep parts of glands and decreased in the isthmus area. Similar patterns of marked changes in CPP32 cell counts were not observed in the gastric mucosa.

In the biopsy samples of the gastric mucosa colonized by *H. pylori* (Groups I and II), the average proliferation index (Ki67 marker) and average apoptosis index (CPP32 marker) were higher, both in the antral and corpus regions of the stomach, compared with the biopsy samples with an unchanged gastric mucosa (Group III; Table 4).

Production of the Bcl-2 marker was not detected in the epithelial cells of the gastric mucosa in either the antral or body regions in any of the examined groups. The Bcl-2 marker was exclusively identified in the cells of the inflammatory infiltrate of the lamina propria of the gastric mucosa.

In 3 biopsies from Group I, the CDX-2 marker (intestinal epithelium transcription factor) was detected within the areas of complete intestinal metaplasia in nearly all goblet-shaped and columnar cylindrical cells. In these areas, the production of intestinal mucin MUC2 was also identified, indicating a disruption in cell differentiation within the epithelial milieu.

Production of MUC5AC protein in biopsy specimens of the antral mucosa and gastric body from Groups I and II was detected in almost all glandular epithelium cells in 28% of cases of the cells in the peristernal zone and 8% of the cells at the bottom of the glands. This mucous membrane distribution was not different from biopsy samples with an unchanged gastric mucosa from Group III. The production of MUC5AC in all biopsies of the gastric mucosa from the antral and body regions in Group III was semiquantitatively assessed as moderate or strong, whereas in the Group II biopsies, it was predominantly evaluated as moderate or weak. In the biopsies of Group II with erosive-ulcerative lesions

Table 3. *Helicobacter pylori* strain genotypes isolated from gastric mucosal biopsies

No	*Helicobacter pylori* Genotypes	I Group (n=44)	II Group (n=38)	In total (n=82)
1	vacAsl/mL; cagA+	1	2	3
2	vacAsl/mL; cagA+; iceAl	0	1	1
3	vacAsl/mL; cagA+; babA2; iceAl	0	1	1
4	vacAsl/mL; cagA−; iceAl	1	1	2
5	vacAsl/mL; cagA−; iceA2	1	0	1
6	vacAsl/m2; cagA+; babA2	2	2	4
7	vacAsl/m2; cagA+; iceAl	1	2	3
8	vacAsl/m2; cagA+; babA2; iceA2	2	1	3
9	vacAsl/m2; cagA−	1	3	4
10	vacAsl/m2; cagA−; iceA2	4	3	7
11	vacAs2/mL; cagA+	1	0	1
12	vacAs2/mL; cagA+; babA2	1	0	1
13	vacAs2/mL; cagA+; iceAl	1	1	2
14	vacAs2/mL; cagA+; babA2; iceAl	0	1	1
15	vacAs2/mL; cagA−	5	2	7
16	vacAs2/mL; cagA−; iceA2	2	1	3
17	vacAs2/m2; cagA+; babA2	1	2	3
18	vacAs2/m2; cagA+; iceAl	2	3	5
19	vacAs2/m2; cagA+; iceA2	2	2	4
20	vacAs2/m2; cagA−	4	2	6
21	vacAs2/m2; cagA−; iceAl	5	3	8
22	vacAs2/m2; cagA−; iceA2	7	5	12

Table 4. Average proliferation index (IP, nuclear label Ki67) and apoptosis index (IA, perinuclear or cytoplasmic label CPP32) of gastric mucosa epithelial cells during infection with several pathogenic *H. pylori* strains

Indexes	Group I (n=44)	Group II (n=38)	Group III (n=32)	p (group I and group III)	p (group II and group III)
Antral region					
IP	28.6 ± 5.2	32.0 ± 5.2	20.3 ± 3.0	0.028	0.008
IA	14.1 ± 2.7	27.3 ± 4.8	6.4 ± 2.5	<0.001	<0.001
Body of the stomach					
IP	21.6 ± 4.3	22.5 ± 2.9	18.6 ± 3.2	0.042	0.036
IA	10.1 ± 3.1	10.3 ± 4.0	5.6 ± 1.9	<0.001	<0.001

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in the antral region, the production of this mucin was slightly reduced; however, no significant differences were observed when compared with Group I (Table 5).

The MUC6 (gastric acid mucin) tag was located in gastric mucosa biopsy samples colonized by bacteria (Groups I and II) in the lower glands. On the other hand, it was detected it in the middle and lower third of the glands, including the basal sections, as well as in the parasternal area in biopsy samples with intact mucous membranes. The production of MUC6 in all biopsies of Groups I and II was semiquantitatively assessed as moderate or weak, with no significant differences observed between the groups (Table 5).

Production of Shh, a factor regulating differentiation of the gastric epithelium, was detected in all epithelial differon cells of the anterior mucosa and gastric body. However, in contrast to the group with an unchanged mucosa, more pronounced expression of Shh protein was noted in gastric epithelial cells (production index 2 vs 8) in biopsy specimens of the gastric mucosa during colonization by bacteria (Groups I and II; Table 5).

Lymphoepithelial interactions in the gastric mucosal epithelium during interaction with **H. pylori** *in the context of host cytokine gene polymorphism*

Examination of the polymorphic locus C-511 T of the IL-1ß gene revealed a statistically significant higher frequency of the T allele (χ^2=13.648; p=0.003, OR=1.1276; 95% CI=1.38–2.88) and T/T genotype (χ^2=6,923; p=0,009, OR=1.1358; 95% CI=1.26–2.45) in patients with *Helicobacter* infection compared with the control (Group IV; Table 6).

Analysis of the genotyping data of IL-1ß gene polymorphism (C +3953 T) and VNTR (variable-number tandem repeats) polymorphism in the 2nd intron of the IL-IRN gene showed that the distribution of genotypes and alleles of these genes did not differ in the *H. pylori*-infected group from that of the control (Group IV). In the patients with *Helicobacter* infection and the control group, the C allele of the IL-1ß gene (C +3953 T) significantly predominated. Among the genotypes, the C/C and C/T (Table 6) were more frequently observed. The allele with 4 repeats of the IL-1RN gene was detected in 83–85% of the patients, while the allele with 2 repeats was found in 48–52% of the patients. The other alleles with 3, 5, and 6 repeats were present in less than 5% of the patients. The most common genotype was 4R/4R (Table 7).

Comparison of the data from the morphological and molecular genetic studies revealed that a weak inflammatory reaction in the duodenal plate was found significantly more frequently in carriers of the C/C gene of IL-1ß (C -511 T; χ^2=9.964; p=0.002). A moderate degree of infiltration by lymphocytes and macrophages was detected in C/T genotype carriers (χ^2=21.738; p=0.001). A pronounced infiltration of immunocompetent cells was predominantly found in T/T genotype carriers (χ^2=18.782; p=0.002). In addition, a statistically significant higher frequency of severe mucosal inflammation was more frequently detected in T allele carriers compared with C allele carriers (χ^2=3.682; p=0.026). This suggests an association of the T allele with a more severe course of chronic inflammation (OR=2.9773; CI=1.19–8.12). Phenotypically, lymph ocyte-macrophage infiltrate cells were represented predominantly by CD20+, CD4+, and CD8+ lymphocytes. Lymphoid nodules were found in the laminas of carriers of this allele in the majority of gastric mucosal biopsy samples.

At the same time, another polymorphism of the IL-1ß gene (C 3953 T) was not related to the severity of the inflammatory

Table 5. Semiquantitative assessment of mucin and Shh production in the antral region and body of the stomach

	Group I (n=44)	Group II (n=38)	Group III (n=32)
Production from the antral region			
MUC5A	moderate	weak	moderate and strong
MUC6	moderate and weak	moderate and weak	moderate
Shh (production index)	6	6	2
Production from body of the stomach			
MUC5A	moderate and strong	moderate and weak	moderate and strong
MUC6	moderate and weak	moderate and weak	moderate
Shh (production index)	6	8	2

Expression of MUC5AC, MUC6 according to the degree of staining: weak: staining of up to 30% of cells, moderate: staining of 31–60% of cells, strong: staining of 60% or more cells. The Shh production index: coloration intensity index (1: weak, 2: moderate, and 3: severe) × the percentage of positively colored cells of the total number (>500) of epithelial cells (0: up to 5%, 1: 5–25%, 2: 26–50%, 3: 51–75%, 4: 75% and more).

Table 6. Frequency of genotype occurrence at the polymorphic loci C -511 T and C +3953 T of the IL-1β gene in patients with *Helicobacter* infection and the control (Group IV)

Genotype	Patients with *Helicobacter* infection (n=41)	Control group IV (n=32)
C −511T		
T/T	9/41 (22.0%)	3/32 (9.3%)
C/T	13/41 (31.7%)	7/32 (21.9%)
C/C	19/41 (46.3%)	22/32 (68.8)%
C +3953T		
T/T	11/41 (26.8%)	7/32 (21.9%)
C/T	13/41 (31.7%)	10/32 (31.3%)
C/C	17/41 (41.5%)	15/32 (46.8%)

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Table 7. Frequency of genotype occurrence at the polymorphic locus VNTR-intr2 of the IL-1RN gene in patients with *Helicobacter* infection and the control (Group IV)

Genotype	Patients with *Helicobacter* infection (n=41)	Control group IV (n=32)
2R/2R	5/41 (12.2%)	4/32 (12.5%)
2R/4R	14/41 (34.1%)	13/32 (40.6%)
4R/4R	20/41 (48.8%)	14/32 (43.8%)
Other alleles with 3, 5, 6 repeats	2/41 (4.9%)	1/32 (3.1%)

response in the intrinsic lamina of the gastric mucosa. In most carriers of both the C and T alleles, moderate infiltration of the intrinsic lamina by lymphocytes and macrophages was observed (52 and 57%, respectively). In addition, no associations or alleles of the IL-IRN gene's polymorphic locus were found with the same intensity as the inflammatory response. Most carriers of 4R and 2R alleles had moderate infiltrations of immunocompetent cells in gastric mucous membranes (54% and 61%, respectively). The most significant indices of structural changes of local immunity were noted in those who were infected with aggressive strains of *H. pylori* (cagA+ and babA2+) and had a proinflammatory host genotype (IL-1ß -511 *T). Thus, carriers of the T allele of the polymorphic locus C-511 T of the IL-1ß gene had an increased number of cells with the CD20+, CD4+, and CD8+ phenotypes together with the highest values for the area of lymphoid nodules with light centers. In the carriers of the C allele of the C-511 T polymorphic locus of the IL-1ß gene, lymphoid nodules were virtually absent. A moderate number of CD20, CD4, and interepithelial cells (CD8) were present in most cases in the generative area.

***Lymphoepithelial interactions in the gastric mucosal epithelium following successful eradication of* H. pylori**

After eradicating *H. pylori*, the gastric mucosa showed good restorative ability. In the early (1–2 months) and late (12 months) terms of the recovery period, the lymphocyte-macrophage infiltrate density gradually decreased. Furthermore, disappearance of the lymphoid nodules in the intrinsic lamina of the gastric mucosa and restoration of epithelium cell renewal parameters were observed. However, not all patients had a favorable recovery period dynamic.

In 74.0% of the biopsy specimens obtained from patients 12 months after eradication of the infection, minimal changes were detected in the gastric mucosa (regression of the cellular infiltrate). In 26.0% of the biopsy specimens taken 12 months later, the high cell density of the native lamina of the antral region was preserved, as was a reduced CD4+/CD8+ lymphocyte ratio (immunoregulatory index, 1.02 ± 0.2). This was regarded as an unfavourable course of the recovery period. In 9.8% of cases, lymph nodes with luminous centers remained in the intrinsic lamina. Inter-epithelial lymphocytes were detected with prevalences according to the volume fractions of these structures in the glandular isthmus.

At 12 months after treatment, the proliferation and apoptosis indices of immunocompetent cells in patients with retained native mucosal infiltrates remained significantly higher compared to similar parameters with an unchanged mucosa without *H. pylori* infection (19.7 ± 5.3 and 6.7 ± 2.3 vs. 26.2 ± 5.3 and 11.6 ± 2.8 respectively, $p<0.05$). Analysis of *H. pylori* genotyping in biopsy specimens with regression and preservation of cellular infiltrates 12 months after treatment revealed no statistical differences in the distribution of *H. pylori* strains.

Examination of the polymorphic locus *C-511 T* of the *IL-1ß* gene revealed a statistically significant increase in the frequency of the *T* allele in patients with preserved cellular infiltrates. In other patients, minimal changes were observed in the gastric mucosa in biopsy specimens one year after eradication (χ^2=3.874; p=0.036; OR=1.721; 95% CI=1.12–3.15). A genetic study of *IL-1ß* gene polymorphism (C +3953 T) showed that carriers of the *T* allele were also statistically significantly more likely to have an unfavourable recovery period (χ^2=4.145; p=0.028, OR=2.0134; 95% CI=1.11–3.28). When studying the VNTR polymorphism in intron 2 of the *IL-1RN* gene, no association was found between the allele genotype and cell infiltration retention in the intrinsic lamina of the gastric mucosa following the elimination of infection. Analysis of alleles in combination revealed that an unfavourable course of the recovery period was characterized by carriage of mainly the *IL-1ß -511*T/IL-1RN*2* and *IL-1ß +3953*T/IL-IRN*2* genes. It was found that after eradication, patients with preserved cellular infiltrates in their gastric mucosa plates were carriers mainly of IL-1ß*T/IL-1RN*2R haplotypes after 12 months. It was also found that they were equally infected with both aggressive strains (cagA+ and BabA2+) and other strains of *H. pylori.*

DISCUSSION

Under the conditions of the interaction of two functional systems of organisms—the gastric mucous membrane epithelium and *H. pylori* and its genetic strains, this study revealed permanent proliferation and apoptosis of the epithelium, expansion of the proliferative compartment, and translocation of the proliferative compartment in the direction of the gastric lumen or gland floor, combined with pronounced apoptosis of the papillary and glandular epithelium.

The study also showed that when infected with *H. pylori*, the Shh protein (traditionally regarded as a marker of gastric epithelial differentiation) is present in cells lining gastric pits and valleys. It is also present in the epithelium of the deep sections of the fundic and pyloric glands. MUC5AC (gastric protector mucin) is produced by the cells of the glandular papillary epithelium and individual cells of the peri-ischemic zone. MUC6 (gastric protector mucin) is localized in the gastric mucosa cells exclusively in the basal part of the glands. The topography of these cells differs from their location in the unchanged mucosa, which reflects changes in the functioning of epithelial differons under the impact of *Helicobacter*.

H. pylori strains are characterized by marked genetic heterogeneity, which may result in various clinical consequences of the persistence of these bacteria [16]. Most people infected

with *H. pylori* have no clinical manifestations of infection [17]. However, colonization of the gastric mucosa by this bacterium can often cause chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and primary B-cell lymphoma [2].

Studies show that pathogenic *H. pylori* strains contain cagA, vacA, iceA, babA, flaA, and flaB genes [28–32]. Observations have shown that patients infected with CagA+ strains of *H. pylori* develop gland atrophy of the gastric mucosa much more frequently [33]. An *H. pylori* strain with the vacAs1 genotype is associated with a high risk of developing a gastric ulcer, atrophic gastritis, and gastric adenocarcinoma [34]. The most significant risk of gastric cancer development was found in patients infected with *H. pylori* possessing the babA gene [35, 36]. Furthermore, the presence of the iceA1 gene is probably a marker of peptic ulcer disease [34, 37].

The present study showed the relationships between the regenerative histogenesis of the gastric mucosa and strain-specific features of *H. pylori*. An increase in the proliferation and apoptosis of the papillary and glandular epithelium of the gastric mucosa is observed when the host is infected with cagA(+) and babA (+) strains of *H. pylori*. A significant increase only in the apoptosis index in the epithelial differon is typical for infection with vacAsl(+) or vacAs2(+) strains of *H. pylori*, with a deficiency of cellular forms of the differon.

The present study shows that structural manifestations of local immunity, when infected with *H. pylori*, are characterized by an absolute increase in the number of immunocompetent cells. Carriers of the *T* allele of the polymorphic locus *C-511 T* of the *IL-1ß* gene had an increased number of cells with CD20+, CD4+, and CD8+ phenotypes. The most significant indicators of mucous immunity structural equivalents listed above were noted in those who were infected with aggressive strains *of H. pylori* (cagA(+) and babA2(+)) and had the proinflammatory host genotype *IL-1ß C -511*T*.

The study showed that independent of the biologic properties of *H. pylori* strains, an unfavourable course of the recovery period (12 months after successful eradication) developed in hosts having a combination of *IL-1ß -511*T/IL- 1RN*2* and *IL-1ß +3953* T/IL-IRN*2* alleles. On the contrary, at 12 months post-eradication in carriers of the *IL-1ß-511C/IL-1RN4* and *IL-1ß+3953C/IL-1RN4* alleles, a transition from reparative epithelial regeneration to physiological was observed.

Thus, the study showed the presence of a proinflammatory haplotype accounting for high and prolonged mucosal immunity in the host, regardless of the *H. pylori* strain with which the host was infected. As the immune system has a morphogenetic function, it is thought that high indices of local immunity will cause more pronounced changes in the cellular renewal of the epithelial differon in individuals with the *IL-1ß *T/IL-1RN*2* genotype. These changes manifest as increased proliferation and apoptosis indices, expansion of proliferative and apoptotic compartments, and translocation towards the gastric lumen or bottom of the glands.

CONCLUSION

The most pronounced changes in indicators of reparative regeneration of epithelial differentiation (increase in proliferation and apoptosis indices) and epithelium differentiation (increased production of the gastric differentiation protein Shh and gastric mucin MUC5AC and decreased production of the protective mucin MUC6 by isthmus gland cells) are noted during colonization of the gastric mucosa by cagA(+) and BabA2 (+) strains of *H. pylori*. The presence of the vacAs1 and vacAs2 genes in *H. pylori* results in a high level of epithelial cell apoptosis without proliferation acceleration. The changes in the epithelial differon were the result of the gastric mucosal response determined by the combination of the *H. pylori* strain products isolated from the infected subject and the level of mucosal immunity determined by the genetic polymorphisms of IL-1ß (C −511 T and C +3953 T) and IL-1RN (VNTR in intron 2) in the host.

After the successful eradication of *H. pylori*, an unfavourable course of the 12-month recovery period develops in hosts having a combination of *IL-1ß -511*T/IL-1RN*2* and *IL-1ß +3953* T/IL-IRN*2* alleles, regardless of the bacterium's biological properties. This is characterized by the preservation of immunocompetent cells in the appropriate lamina of the gastric mucous membrane combined with a high level of renewal in the epithelial differon.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated during and/or analyzed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

All methods were performed according to the principles of the Declaration of Helsinki. The study was approved by the Local Ethics Committees of I.M. Sechenov First Moscow State Medical University (Sechenov University; Protocol no. 12 of 02.04.2022).

CONSENT TO PARTICIPATE

Informed consent was obtained from all participants.

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