СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА ЦИТОКИНОВОЙ РЕГУЛЯЦИИ У ПАЦИЕНТОВ С ЗАБОЛЕВАНИЯМИ ЖЕЛУДКА, АССОЦИИРОВАННЫМИ С *HELICOBACTER PYLORI*-ИНФЕКЦИЕЙ

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Резюме. При инфицировании организма бактерией *Helicobacter pylori* запускается цитокиновый каскад, играющий ключевую роль в прогрессировании хронических воспалительных и деструктивных процессов в слизистой оболочке желудка. Таким образом, происходит стимуляция секреции целого ряда цитокинов, которые в свою очередь способствуют привлечению иммунокомпетентных клеток, развитию воспалительных изменений. Однако гиперпродукция цитокинов может привести к атрофическим изменениям СОЖ и как следствие перерождение в рак желудка. Таким образом, роль цитокинов в предраковых состояниях неоднозначна, с одной стороны, они активируют иммунный ответ, направленный на элиминацию патогена, с другой, сами способствуют прогрессированию заболевания.

В комплексное клинико-лабораторное исследование были включены больные: 60 – с хроническим гастритом (ХГ), 55 – с хроническим атрофическим гастритом (ХАГ), 50 – с раком желудка (РЖ, I-II стадии, морфологический вариант – аденокарцинома) и 60 – контрольная группа. Диагнозы верифицировались согласно международным и Российским рекомендациям и подтверждались лабораторно-инструментальными исследованиями. Все больные были сопоставимы по гендерно-возрастным характеристикам ($p > 0,05$). У всех больных выявлялись специфические IgG к *H. pylori*. Исследование было одобрено локальным этическим комитетом ФИЦ КНЦ СО РАН (протокол № 11 от 11.11.2013), соблюдались все этические требования, больные подписывали форму информированного согласия на участие. У больных и лиц контрольной группы проводился однократный забор крови из локтевой вены при поступлении в вакутейнеры с гепарином.

Уровни IL-2, IL-4, IL-8, TNF-α, интерферона-γ в сыворотке крови больных и здоровых лиц определяли с помощью метода иммуноферментного анализа с использованием наборов реагентов производства АО «Вектор-Бест». Статистическая обработка данных проводилась с помощью пакетов прикладных программ Statistica for Windows 8.0.

У всех больных с *H. pylori* – ассоциированными заболеваниями (ХГ, ХАГ, РЖ) выявляется увеличение провоспалительных (IL-2, IL-8, IFNγ) со значительным ростом IL-8 у всех больных и IFNγ при РЖ и противовоспалительного цитокина (IL-4) с максимальным значением при раке желудка. Обнаруживается сочетанный Th1- и Th2-опосредованный иммунный ответ с максимальным нарушением цитокиновой регуляции при РЖ.

Ключевые слова: интерлейкины, Helicobacter pylori, хронический гастрит, хронический атрофический гастрит, рак желудка, иммунитет

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COMPARATIVE CHARACTERISTICS OF CYTOKINE REGULATION IN PATIENTS WITH GASTRIC DISEASES ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION

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Abstract. When the body is infected with the bacterium *Helicobacter pylori*, a cytokine cascade is launched, which plays a key role in the progression of chronic inflammatory and destructive processes in the gastric mucosa. Thus, the secretion of a number of cytokines is stimulated, which in turn contribute to the attraction of immunocompetent cells and the development of inflammatory changes. However, hyperproduction of cytokines can lead to atrophic changes in the gastric mucosa and, as a result, degeneration into gastric cancer. Thus, the role of cytokines in precancerous conditions is ambiguous. On the one hand, they activate the immune response aimed at eliminating the pathogen. On the other hand, they themselves contribute to the progression of the disease.

The complex clinical and laboratory study included patients: 60 with chronic gastritis (CG), 55 with chronic atrophic gastritis (CAG), 50 with gastric cancer (GC, stage I-II, morphological variant – adenocarcinoma) and 60 – control group. The diagnoses were verified according to international and Russian recommendations and confirmed by laboratory and instrumental studies. All patients were comparable in terms of gender and age characteristics ($p > 0.05$). All patients had specific IgG to *H. pylori*. The study was approved by the Local Ethics Committee of the FRC KSC SB RAS (protocol No. 11 dated November 11, 2013). All ethical requirements were observed, and the patients signed the informed consent form for participation. Patients and persons of the control group underwent a single blood sampling from the cubital vein upon admission to vacutainers with heparin.

The levels of IL-2, IL-4, IL-8, TNFα, interferon-γ in the blood serum of patients and healthy individuals were determined using the enzyme immunoassay method using reagent kits manufactured by JSC "Vector-Best". Statistical data processing was carried out using the Statistica for Windows 8.0 application package.

All patients with *H. pylori*-associated diseases (CG, CAG, GC) showed an increase in pro-inflammatory (IL-2, IL-8, IFNγ) with a significant increase in IL-8 in all patients and IFNγ in gastric cancer and anti-inflammatory cytokine (IL-4) with a maximum value in gastric cancer. A combined Th1 and Th2 is found – a mediated immune response with a maximum violation of cytokine regulation in gastric cancer.

Keywords: interleukins, Helicobacter pylori, chronic gastritis, chronic atrophic gastritis, stomach cancer, immunity

Introduction

Currently, *H. pylori* infection is classified as a carcinogen, and long-term persistence of infection in the gastric mucosa initiates a cascade of pathogenetic disorders from inflammatory changes to atrophy and even metaplasia of gastric epithelial cells [2]. The number of patients with diseases of the stomach increases annually, while even in practically healthy volunteers, infection of the gastric mucosa with a pathogenic bacterium is found. All this determines the relevance of studying this topic [1, 3, 4, 6]. **The aim of our work** was to compare the content of cytokines and study the features of cytokine regulation in chronic gastritis, chronic atrophic gastritis and gastric cancer infected with *H. pylori*. We assume that a common pathogenetic mechanism caused by *H. pylori* infection will be revealed in different pathogenetic diseases.

Materials and methods

A comprehensive clinical and laboratory study included patients: 60 with chronic gastritis (CG), 55 with chronic atrophic gastritis (CAG), 50 with stomach cancer (SC, stages I-II, morphological variant – adenocarcinoma) and 60 – control group. The diagnoses were verified according to international and Russian recommendations and confirmed by laboratory and instrumental studies. All patients were comparable in terms of gender and age characteristics ($p > 0.05$). All patients had specific IgG to *H. pylori*. The study was approved by the Local Ethics Committee of the FRC KSC SB RAS (protocol No. 11 dated November 11, 2013). All ethical requirements were observed, and the patients signed the informed consent form for participation. Patients and persons of the control group underwent a single blood sampling from the cubital vein upon admission to vacutainers with heparin.

The content of cytokines was determined in blood plasma by enzyme immunoassay using a Multiskan FC (Thermoscientific) analyzer. Based on the analyses, a database was compiled in Excel, and statistical processing was carried out in the Statistica 8.0 program [5, 7, 8].

Results and discussion

In patients with CG, CAG and SC there is an increase in the content of IL-2 compared with the control group, with the maximum value of the indicator in CG (Table 1). In patients of all the studied groups, there is a significant increase in the median

concentration of IL-8 compared with practically healthy volunteers, with the maximum value of the median parameter in SC. The median of the indicator increases by about 20 times in all the studied patients. In all patients, there is a significant increase in the median content of IFNγ relative to the control, 5 times in patients with CG and CAG and 8 times in patients with SC.

Thus, in all patients with inflammatory changes in the gastric mucosa (chronic gastritis), degenerative-inflammatory processes (chronic atrophic gastritis) and with metaplasia of epithelial cells of the gastric mucosa (cancer of the stomach), an increase in the content of pro-inflammatory cytokines (IL-2, IL-8, IFNγ) with a significant increase in IL-8 in all patients and IFNγ in stomach cancer.

The content of anti-inflammatory cytokine (IL-4) was assessed in CG, CAG and SC. There is a large increase in the median concentration of IL-4 in all patients; the median content of IL-4 is approximately increased by 13 times with a maximum level of IL-4 in stomach cancer.

Thus, in all patients there is an increase in pro-inflammatory and anti-inflammatory cytokines, which indicates the activation of immune cells and an imbalance in the cytokine regulation system. An increase in the production of Th1 and Th2 helper cytokines indicates a combined Th1- and Th2-mediated immune response in *H. pylori*-associated diseases (CG, CAG, and SC).

At the next stage, the immune responses in CAG were characterized depending on the severity of atrophy relative to the control group, patients with CG and SC. In general, regardless of the severity of degenerative-atrophic changes in the gastric mucosa, there was an increase in pro-inflammatory cytokines (IL-2, IL-8, IFNγ) and anti-inflammatory cytokine (IL-4) relative to the control group, while there was a tendency to increase medians of all indicators in severe atrophy of the gastric mucosa.

When comparing the parameters in CAG depending on atrophy relative to those in CG and SC, no statistically significant changes in the parameters of pro- and anti-inflammatory cytokines were obtained, while the trend towards an increase in the median of all cytokines in severe CAG versus CG and in SC versus severe CAG remains. Taking into account that all patients with CG, CAG and SC had specific antibodies to *H. pylori*, in this case we regard all conditions as *H. pylori*-associated diseases. The inflammatory process in the cancer of the stomach is

TABLE 1. CONTENT OF CYTOKINES IN PATIENTS WITH CHRONIC GASTRITIS (CG), CHRONIC ATROPHIC GASTRITIS (CAG), GASTRIC CANCER (GC) COMPARED WITH THE CONTROL GROUP (Me, $Q_{0.25}$-$Q_{0.75}$, p_{M-U})

Indicator	Control group, n = 60 (1)		Patients with CG, n = 60 (2)		Patients with CAG, n = 55 (3)		Patients with CAG PI 25 -50 μg/L, n = 24 (4)		Patients with CAG PI < 25 μg/l, n = 31 (5)		Patients with GC, n = 50 (6)	
	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$	Me	$Q_{0.25}$-$Q_{0.75}$
TNFα (pg/mL)	0.54	0.38-0.87	0.67	0.44-0.93	0.78	0.56-1.30	0.7	0.54-1.20	0.78	0.56-1.30	0.76	0.45-0.95
IL-2 (pg/mL)	1.1	0.50-3.05	5.7	3.6-10.3	4.9	3.8-9.5	4.4	3.8-10.1	4.9	3.8-9.5	5.2	3.0-8.7
			$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-4} < 0.001$	
IL-8 (pg/mL)	2.1	0.5-4.0	40.5	7.5-97.2	38.1	5.5-87.3	37.3	4.2-85.6	38.1	5.5-87.3	41.1	12.2-99.5
			$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-4} < 0.001$	
IFNγ (pg/mL)	0.6	0.22-4.00	2.9	2.2-4.0	3.2	2.3-4.8	3.1	1.9-5.1	3.2	2.3-4.8	4.4	3.3-6.9
			$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-4} < 0.001$; $p_{2-4} = 0.02$	
IL-4 (pg/mL)	7.0	5.6-7.8	86.8	76.8-103.5	91.4	73.2-112.3	88.7	68.2-105.4	91.4	73.2-112.3	93.3	68.6-122.1
			$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-2} < 0.001$		$p_{1-3} < 0.001$		$p_{1-4} < 0.001$	

Note. p_{1-2}, statistically significant differences between the group of CG patients and the control group; p_{1-3}, statistically significant differences between the group of patients with CAG and the control group; p_{1-4}, statistically significant differences between the control group and the group of patients with CAG with a PI level of 25-50 μg/L; p_{1-5}, statistically significant differences between the control group and the group of CAG patients with PI < 25 μg/L; p_{1-6}, statistically significant differences between the group of patients with gastric cancer and the control group; p_{2-3}, statistically significant differences between the group of patients with CG and the group of patients with CAG; p_{2-4}, statistically significant differences between the group of patients with CG and the group of patients with CAG with a PI level of 25-50 μg/L; p_{2-5}, statistically significant differences between the group of patients with CG and the group of patients with CAG with PI < 25 μg/L; p_{4-5}, statistically significant differences between the group of patients with CAG with a PI level of 25-50 μg/L and the group of patients with CAG with a level of PI < 25 μg/L; p_{2-6}, statistically significant differences between the group of patients with chronic hepatitis and the group of patients with GC; p_{3-6}, statistically significant differences between the group of patients with CAH and the group of patients with GC; p_{4-6}, statistically significant differences between the group of patients with CAH with a PI level of 25-50 μg/L and the group of patients with GC; p_{5-6}, statistically significant differences between the group of patients with CAH with PI < 25 μg/L and the group of patients with gastric cancer;

initiated by *H. pylori*, followed by other pathogenetic changes, such as atrophy and metaplasia.

The criterion for inflammation according to a clinical blood test is leukocytosis. We studied the correlation relationships between leukocytosis and indicators of pro-inflammatory and anti-inflammatory cytokines in control, in chronic hepatitis, CAG and SC. The following relationships were obtained: in the control group, five to four straight lines between the number of leukocytes and the content of TNFα ($r = 0.7$; $p = 0.05$), IFNγ ($r = 0.76$; $p = 0.01$), IL-2 ($r = 0.84$; $p = 0.015$), IL-8 ($r = 0.88$; $p = 0.02$) and inverse with IL-4 ($r = -0.64$; $p = 0.03$), which is natural, since pro-inflammatory cytokines increase leukocytosis, anti-inflammatory cytokine reduces the number of leukocytes. In patients with CG and CAG, 4 direct correlations are found: IFNγ (CG – $r = 0.81$; $p = 0.013$, CAG – $r = 0.69$; $p = 0.04$), IL-2 (CG $r = 0$ .8; $p = 0.015$, CAG – $r = 0.71$; $p = 0.02$), IL-8 (CG – $r = 0.82$; $p = 0.04$, CAG – $r = 0.9$; $p = 0.01$) and inverse with IL-4 (CG – $r = -0.6$; $p = 0.05$, CAG – $r = -0.7$; $p = 0.02$), there is no connection with TNFα, and with stomach cancer there are only 3: direct – IFNγ ($r = 0.72$; $p = 0.03$), IL-8 ($r = 0.89$; $p = 0.02$) and inverse with IL-4 ($r = - 0.78$; $p = 0.01$), TNFα and IL-2 exist autonomously.

All patients with *H. pylori*-associated diseases (CG, CAG, SC) showed an increase in pro-inflammatory (IL-2, IL-8, IFNγ) with a significant increase in IL-8 in all patients and IFNγ in stomach cancer and anti-inflammatory cytokine (IL-4) with a maximum value in cancer of the stomach. A combined Th1 and Th2 is found – a mediated immune response with a maximum violation of cytokine regulation in stomach cancer.

Conclusion

Correlation analysis confirmed the pathogenetic significance of the parameters IL-8, IL-2, IFNγ, IL-4 for CG and CAG, and IL-8, IFNγ, IL-4 for SC. Despite the different diseases, a single common pathogenetic mechanism caused by *H. pylori* infection was identified, which proves the need for mandatory eradication of the pathogen when it is detected, even in the absence of clinical symptoms in practically healthy people.

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