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MINIREVIEWS

Helicobacter pylori vs immune system or antibiotics

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Abstract

Helicobacter pylori (*H. pylori*) infection has often no clinical signs and is one of the most common bacterial infections. All infected subjects have histology of active chronic gastritis. In some cases patients develop peptic ulcer and minority of them develop gastric cancer. Gastric cancer is multifactorial disease, thus various progressions of *H. pylori* infection and disease are dependent on the host genetic factors, the characteristics of the individual's immune response, environmental factors, and different bacterial virulence factors of the individual bacterial strains. Eradication of the bacteria plays a crucial role in the treatment of these cases however antibiotic therapy does not always help. Bacteria often develop resistance to antibiotics so we recommend that not only screening for *H. pylori* also the strain determination should have some diagnostic value, especially in the patients who already developed gastritis. Furthermore, for such patients assessment of disease progression (atrophic or metaplastic gastritis) could be followed by polymorphism determination. Until now we cannot predict the disease based only on single polymorphism. Bacteria successfully neutralize the responses of the immune systems using different enzymes or even components of the host immune response. However, the influence of immune system and its components could represent new ways of treatments and could help to eradicate the infection.

Key words: *Helicobacter pylori*; Resistance to antibiotics; Immune response; Genetic factors; Bacterial eradication

Core tip: Combination of *Helicobacter pylori* (*H. pylori*) and host-associated risk factors do not always allow evaluation of gastric carcinoma. We have learnt that the assessment of patients with *H. pylori* infection and its strain is very important and concluded that eradication of bacteria has essential meaning. We recommend that not only screening for *H. pylori* also the strain determination should have some diagnostic value, especially in the patients who already developed gastritis. Furthermore, for such patients assessment of disease progression could be followed by polymorphism determination. Conclusions indicate that host cytokine genotypes, host immune response, as well as *H. pylori* strains could be important for greater risk for developing gastric cancer.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is 3-5 μm Gram-negative microaerophilic bacterium. It colonizes the gastric mucosa and metaplastic gastric epithelium of duodenum. It has characteristic spiral morphology with more flagella at one pole and is capable of moving in the protective gastric mucus layer. It has enzymes catalase, oxidase and degrades urea. While living in extremely unfavorable environment of low pH in the stomach, the protection for survival is provided by the clearance of gastric mucus and secretion of proteases that degrade additional mucus, and urease that produces ammonia, which helps to raise the pH in the vicinity of bacteria[1,2].

Epidemiology

H. pylori infection results in chronic active gastritis, and 20% of infected also develop ulcers of the stomach or duodenum. In some cases infection leads to stomach cancer and MALT lymphoma. Although *H. pylori* is not invasive bacteria, the mechanisms of mucosal inflammation and tissue damage and the onset of the disease are a combination of bacterial and host factors[2]. *H. pylori* is a human pathogen, and its reservoir is the human stomach. It colonizes cardio, corpus and antrum of the stomach. The transmission of *H. pylori* from the stomach of one human to another is not yet fully clear. The transfer is most common within families and during childhood. In all likelihood, there are two routes of transmission; the oral-oral route and the fecal-oral route. Fecal-oral route is associated with the ability of bacteria to survive outside its primary environment. For this pathway certain conditions must be met, namely the rapid passage through the intestine, most often in the case of diarrhea, inadequate sanitation and sewage disorder. Furthermore, contamination of drinking water by sewage is also possible. Such conditions are usually found in developing countries where the prevalence of *H. pylori* infection is greater than in developed countries. Oral-oral route is associated with regurgitation of gastric juice and thus passage of bacteria in the mouth and the transmission of *H. pylori* in close contact to another person[3,4]. *H. pylori* infect more than half of the world's population. This bacterium can be left in the gastric mucosa for a long time, without causing disease symptoms including rare spontaneous disappearance from the stomach[3]. The highest infection rate is 54% of the age group 60 to 69 years. In the age group 0-19 years, 10% of those living are infected, and in the age group between 20 and 49 years 29% of the population is infected with *H. pylori*[5].

The pathogenesis and virulence factors

H. pylori has adapted to live between the protective layer of mucus and epithelial cells of the gastric mucosa. The bacterium develops in neutral pH, and is changed to coccoid shape at a pH below 4 or above 8. The gastric mucosa can be damaged directly or indirectly by influencing on the homeostasis of acid secretion[6].

The inner part of the *H. pylori* cell membrane, which is situated at the cytoplasmic membrane, consists of peptidoglycan. On the outer side the outside membrane with anchored lipoproteins is located, which are sometimes covalently bound to the peptidoglycan. The main surface components of Gram-negative bacteria are lipopolysaccharides (LPS), which protect the bacteria from the environment[3]. The basis represents the lipid A of LPS, which allows the biological activity of the endotoxin[3,7]. *H. pylori* has enzyme urease on the outer and inner membrane, which can be excreted in the neighborhood through the membranes of dead cells. It degrades urea to ammonia and bicarbonate, and thus neutralizes the acid environment. The bacterium is able to attach on epithelial cells of the gastric mucosa and moves as a spiral through the mucus in the stomach. The enzymes protease, lipase and phospholipase allow to cleavage the glycoprotein of mucous gel into a more hydrophobic structure, which can cause diffusion of hydrogen ions and possible damage to the mucosa. Ammonia can react with hypochlorous acid of neutrophils resulting in cytotoxic compounds such as monochloramine and hydroxylamine[7].

Genetic information for vacuolization cytotoxin (Vac A) have all *H. pylori*, approximately 50% of them produce 67 kDa protein, which causes the formation of vacuoles in epithelial cells of the gastric mucosa. Cytotoxin-associated gene A (*Cag A*) is the genetic makeup of the 127 kDa protein, which is present in all strains. Bacteria that produce CagA cause pronounced secretion of IL-8 from epithelial cells, resulting in increased mobilization of neutrophils and a higher level of active chronic gastritis. Neutrophils cannot effectively phagocyte bacteria and at quicker rupture of the neutrophils free oxygen radicals are released, which further failures the mucous membrane. Patients infected with Cag A-positive strains have increased risk for the development of duodenal ulcers and gastric cancer[6,8].

H. pylori has a protein which causes the attachment of neutrophils to the endothelial cell lining of the stomach and is called HP-NAP (*H. pylori* neutrophil-activating protein). Different degrees of attachment of neutrophils were observed, which are caused by different activities of this protein in various strains of *H. pylori*, which indicates different levels of expression of this protein as similar to protein Vac A. HP-NAP is located in the cytosol of bacteria and is released during lysis. It binds to the outer surface of the outer membrane, similar as urease. The majority of patients infected with *H. pylori* produce a specific antibody to HP-NAP. Vaccination of mice with the protein HP-

NAP provided protection against infection with *H. pylori*. Protein plays an important role in the immune response to infection with the bacterium. HP-NAP is chemotactic for monocytes and neutrophils, and causes increased expression of β2-integrins on neutrophils and monocytes. HP-NAP works through activation of NADPH oxidase in neutrophils and thereby induces the production of reactive oxygen intermediates Reactive oxygen intermediates. HP-NAP represents a virulence factor that is associated with the pathogenic effects of the *H. pylori* to the site of infection[9].

H. pylori affect the homeostasis of gastric acid secretion through changes in paracrine hormone control system for gastrin and acid secretion. Normally, the release of gastrin leads to acid secretion and lowering the pH below 3 promoting cells D to somatostatin secretion, which in paracrine route inhibits the release of gastrin, and consequently acid secretion. Proteins and fatty food in the duodenum *via* cholecystokinin stimulate the secretion of somatostatin and thereby inhibit further secretion of acid. Patients with duodenal ulcer and infected with *H. pylori*, have increased sensitivity of the parietal cells to gastrin. These patients, when stimulated with food or with gastrin, secrete larger amounts of stomach acid than healthy people. After removal of bacteria, secretion of gastrin normalizes, the amount of acid is reduced by 50%, and completely normalizes only after one year. Infected patients have also reduced secretion of bicarbonate into the duodenum. After removal of the bacteria this is also normalized[6].

Virulence factors CagA and VacA and the development of gastric cancer

Virulence factors CagA and VacA are the most important factors of *H. pylori* in the development of the disease. The CAG-pathogenetic islands (cagPAI) have the information for the type Ⅳ secretory system, which is required for peptidoglycan to enter the cell. CagA phosphorylates cellular proteins, which are known oncogenes[10]. The motive for the phosphorylation of CagA is located within the amino acid motif EPIYA. A larger number of EPIYA-C within CagA damage more cells and more severe course of the disease occurs, especially gastric cancer[11]. CagA after phosphorylation connects to the proteins in the cell, which causes an increase in signal similar to that in the expression of growth factors in the cell. It affects the proliferation and adhesion of cells and the organization of the cytoskeleton[12].

VacA is a protein that forms pores in the cell and causes vacuolization and cell death. It is involved in the presentation of antigen and causes efflux of ions out of the cell due to the impact on the integrity of close connections between cells (tight junction). It is also a potent inhibitor of cell activation of T-lymphocytes *in vitro*[13,14]. *H. pylori* has several versions of the protein VacA. Type VacA s1/m1 is the most cytotoxic. Interesting findings are that CagA positive strains has vast majority of s1 type VacA. Vac A has two versions, i1 and i2. Protein VacA s1/m1 i1 is often present in patients with gastric cancer[15].

The immune response to infection

Chronic gastritis is associated with increased local production of IgG, increased infiltration of the mucous membrane with T cells, and increased expression of HLA class Ⅱ molecules[16,17]. In addition, chronic gastritis is a *H. pylori* infection associated with other diseases, such as acute gastritis, peptic ulcer on duodenum, lymphoma of gastric mucosa and gastric adenocarcinoma, which is divided into intestinal and less common diffuse type[18]. The development of disease stages can be divided into three entities. Simple gastritis caused by infection with bacteria, acid secretion is normal. Duodenal ulcer occurs when infection with the bacterium occurs, antral gastritis is present, and acid secretion is increased. This type of patient is protected from gastric cancer. *H. pylori* chronic gastritis in patients with gastric cancer is more intense in the corpus, also more extensive intestinal metaplasia and atrophy of the mucosa are present. There is not enough acid, so intestinal metaplasia might develop, which leads to the intestinal-type of gastric cancer[19,20].

Infection with *H. pylori* causes an increased concentration of IL-8, IL-1, IL-6 and TNF-α. IL-8 in tissue, which activates neutrophils, results in the epithelial cells, as well as in other cells in the wall of the stomach. Expression is dependent on the adherence and the genotype of the *cagA*. The immune response Th1 is triggered in case of infection by intracellular pathogens and cancer, immune responses Th2 is characterized in infection by extracellular pathogens. In contrast to the expected reaction in the host with *H. pylori* infection, Th1 immune response is particular. Bacteria should choose an immune response by stimulating IL-12, which leads to a Th1 response. Thus stimulated immune system cannot overcome the infection. The cellular immune response and the formation of IgG antibodies which can activate complement cause intense inflammation and further damage to the mucosa. Neutrophils which are attracted and activated by IL-8 are an important component in the development of chronic active gastritis. The result of infection with the same strain of *H. pylori* could be different in the immune response of the different entities[6,21].

Infection of the gastric mucosa, accompanied by a strong neutrophilic infiltration of the mucosa, significantly contributes to the formation of gastritis. Strains of *H. pylori*, which are capable to activate neutrophils, are more common in patients with peptic ulcer than in patients with chronic active gastritis[9].

H. pylori possess number of factors that contribute to the colonization and to bacterial adherence. BabA binds to antigens of Lewis b blood group, which are on the cells of the epithelium, and can contribute to the adhesion of bacteria. Transgenic mice expressing

more Lewis b blood group have had heavier chronic gastritis and have lost several parietal cells[4]. SabA is a protein on the outer membrane of *H. pylori* and binds to a glycoprotein antigen of Lewis x blood group and facilitates adhesion. Similar role has OipA. OipA is on the membrane of *H. pylori* is more pronounced in people who already have precancerous changes. Bacteria survive easier in the epithelial cells, since it is difficult to mechanically removed[22].

The influence of immune system on the success of treatment of H. pylori infection

In addition to *H. pylori* for the development of the disease we must also consider the immune system. T lymphocytes in the gastric mucosa mostly express IFN-γ, which indicates Th1 immune response[23]. In patients who have weakened immune response despite treatment with antibiotics are not healed. T lymphocytes without additional stimulation with antigens of *H. pylori* produce smaller amounts of IL-4 than in patients whose therapy was successful with antibiotics[24]. In the patients who received antibiotic therapy and are cured, T lymphocytes, stimulated by antigens of *H. pylori*, express larger amounts of IFN-γ molecules compared to the patients who do not recover despite receiving treatment against bacteria. In cured patients compared with those which were not cured, an increased expression of IFN-γ and IL-4 was observed, when T-lymphocytes were stimulated with dendritic cells, which have been in contact with *H. pylori* antigen[25].

One of the possible methods for treatment of *H. pylori* infection could be *in vivo* addition of recombinant IFN-γ. In normal humans, the addition of IFN-γ mimics the physiological response to bacterial infection, causing increased expression of FcγRI, which is proportional to the biological activity of IFN-γ on neutrophils and monocytes, which enhances phagocytosis by neutrophils mediated by FcγR. In addition to improved defense in normal humans, recombinant IFN-γ also helps in treating various disorders of immunity[26].

It was also found that IL-12 produced by the antigen-presenting cells (APC), in the *H. pylori* infection effected naive CD4-T-lymphocytes, which are directed towards a Th1 response through the transcription factor T-bet and STAT4. This suggests that the infection with *H. pylori* is an important stimulus for the secretion of IL-12[27].

Moreover, IL-18 affects the production of IFN-γ released by T-lymphocytes and NK cells. If the mucosa is infected with *H. pylori*, the regulation of IL-18 expression is influenced by CagA and OipA. Epithelial cells produce IL-18 under the influence of two virulence factors, while monocytes produce more IL-18 only under the influence of OipA. These data confirm the importance of IL-18 in the development of gastritis due to *H. pylori* infection[28]. Th17 T cells could also play an important role in infection with *H. pylori*. It is anticipated that IL-17 plays an important role in mucosal immunity. The expression of IL-17 in the case of *H. pylori* infection is affected by IL-23. If this path is blocked, the infected mucosa has less IL-17[29,30].

APCs have on their membranes' receptors that recognize PRR (Pattern Recognition Receptors), which include Toll-like receptors (TLR). These are specific receptors, which are involved in the mechanisms of innate immunity and can trigger a series of defense mechanisms, such as complement activation, phagocytosis and expression of genes associated with inflammation. TLR receptors recognize conserved structure of microorganism, which are relatively stable within certain groups of microbes and are called Pathogen-Associated Molecular Pattern or PAMP[31]. TLRs recognize different molecular components of microorganisms. Examples of ligands are LPS from the cell wall of Gram-negative bacteria, peptidoglycan, lipoproteins and lipopeptide from the cell wall of Gram-positive bacteria. TLRs detect some bacterial proteins such as flagellin, and a foreign nucleic acid. LPS was the first detected ligand for the TLR, which is recognized by TLR-4. TLR-4 is part of the lipopolysaccharide receptor CD14, and for recognition binding of the complex of LPS and LPS binding protein (LBP) to the receptor CD14 is required[32,33].

On the surface of the membrane of Gram-negative bacteria are LPS, which protect bacteria against bile salts, hydrophobic antibiotics and complement activation. LPS after release organize in aggregates. Using protein LBP a complex LPS-LBP is formed. This binds to the membrane protein CD14 (mCD14), which is located on monocytes, or a soluble CD14 protein (sCD14) which is present free in the serum[31]. LPS of *H. pylori* (Hp LPS) is, compared to the other Gram-negative bacteria, poor immunogen. In addition, Hp LPS as compared to other bacteria binds poorly to the TLR-4 on epithelial cells. TLR-2 and TLR-5 are more important for the innate immune response of epithelial cells on *H. pylori*[34,35]. Due to the poor immunogenicity of Hp LPS scientists began to look for other receptors, which participated in the initial process of the immune response. They found that an important role play *H. pylori* peptidoglycans, which are an important link in the development of mucosal immunity. Peptidoglycan enters the epithelial cell using the type Ⅳ secretory system, which genetic information is located in cagPAI of *H. pylori*. Inside the cell it binds to the NOD-like receptor, which ultimately increases the kinase activity of NF-κB, which activates cell proliferation, and then through the other signals the activation of the immune system[35].

Dendritic cells (DC) play an important role in directing the immune response. *H. pylori* is presented to other immune cells, especially T lymphocytes, and is involved in their activation. In what way DC focus the immune response is not yet fully understood. We know that the response of DC depends on the virulence factors of *H. pylori* and the host immune competence[16]. DC can direct differentiation of T lymphocytes into Th1 subsets, with the consequent emergence of severe gastritis, or subsets Th2, which causes lighter inflammation. Th2 response, in the case of *H. pylori* infection, is less

frequent than Th1. The differentiation in regulatory T-lymphocytes is also possible, which limits the immune response and thereby prevents the formation of more severe forms of inflammation, what helps *H. pylori* to survive[17,36]. DCs which present antigens of *H. pylori*, strongly activate T-lymphocytes, influence on the production of cytokines and initiate an inflammatory process. *H. pylori* LPS (Hp LPS) stimulate DC *via* TLR[37]. TLR are less important for the immune response in the case of epithelial cells, however the APC use TLR for the immune response[38]. DC, together with the cytokines and costimulatory molecules, affect other inflammatory cells, especially the T lymphocytes. Measurement of the immune response in case of infection with *H. pylori* could help to assume in which patients standard therapy with antibiotics are more likely to be ineffective[25].

The influence of host factors on the development of gastric cancer

Several host genetic factors are important for the progression and development of gastric cancer. Single nucleotide polymorphisms or point mutations in genes for cytokines affect gastric acid secretion and innate immune response[39-42]. Polymorphisms in genes may influence the level of the cytokine production, and consequently influence the disease outcome[43].

IL-1β is mainly secreted in response to *H. pylori* infection. It has a proinflammatory activity and strongly inhibits gastric acid secretion[44]. Inhibition of acid secretion leads to the spread of bacteria from the antrum to the corpus, and consequently the development of corpus predominant gastritis which further leads to the development of gastric cancer[45,46]. Three polymorphisms were described in the *IL-1B* gene at positions -31, -511 and +3954 from the transcription start site[45,47]. *IL-1B*-31*C and *IL-1B*-511*T alleles are associated with hypochlohydria or decreased acidity in the stomach in response to the *H. pylori* infection[45]. IL-1β receptor antagonist polymorphism (IL-1ra) has also been associated with the level of IL-1β secretion. Genotype *IL-1RN**2 is associated with higher secretion of IL-1β, most probably through the reduction of its receptor antagonist IL-1ra[47,48].

TNF-α as a central mediator of the immune response has several polymorphisms in the promoter region of *TNF-A* gene of which -308*G > A was associated with increased production of TNF-α in response to the infection, and increased risk of gastric cancer[49-51]. El-Omar *et al*[52] and Machado *et al*[53] found that patients with this polymorphism have almost two-fold increased risk of gastric cancer.

At the position +896, in exon 4 of the *TLR-4* gene a functional polymorphism has been described. This A > G transition results in an alteration of the extracellular domain of TLR-4, that causes hyporesponsiveness to LPS, reduced epithelial TLR-4 density and exaggerated inflammatory cytokine response[54]. A recent studies have reported an association of *TLR-4* gene polymorphisms with gastroduodenal diseases such as gastric atrophy, hypochlorhydria and noncardia gastric cancer[55-58].

Furthermore, our results on Slovenian population showed that males were more predominant to develop gastric cancer than females. Meanwhile females had 2-fold greater probability to develop chronic gastritis[59]. We also proved that *IL-1B*-511*C homozygote allele was most frequent in chronic gastritis group (58.8%). Such results were not found in any other study. According to our findings, individuals carrying the *IL*-1B-511*T/T allele, both homozygotes and heterozygotes, compared to control group showed an increased OR for gastric cancer. Moreover, no indications that the infection with *H. pylori* in a given inflammatory genotype could result in an inflammatory response, and then gastritis or cancer could be found[59]. *TLR-4* or *TNF-A* polymorphism did not play a role in the development of gastric premalignancies. The results were comparable to those by Garza-Gonzales *et al*[55] and confirmed in review by Figueiredo *et al*[60] for TLR-4. Meanwhile, in 2015 Trejo-de la *et al*[61] suggested that 2848G > A polymorphism in *TLR-9* increased the risk for the development of duodenal ulcer.

Treatment of H. pylori infection

In addition to the immune response, which is difficult to influence, an appropriate antibiotic therapy is important. Infection with *H. pylori* is most effectively cured with a proton pump inhibitor (PPI) and a combination of two antibiotics. We mainly use metronidazole and clarithromycin or clarithromycin and amoxicillin or amoxicillin and metronidazole. Certain strains of *H. pylori* became resistant to metronidazole and clarithromycin. However, with antibiotics we cannot eradicate *H. pylori* in about 10% of patients in whom the bacterium is not resistant to selected antibiotics[62-64]. If we want to successfully treat the *H. pylori* infection, we need to know the primary resistance of *H. pylori* to antibiotics. Less developed regions have a very high resistance to clarithromycin and metronidazole, in the case of metronidazole ranging up to 100%[64]. Indications for treatment and methods of treatment are set out in national and international guidelines. The success of the treatment decreases with age, and the 7-d treatment in the United States is between 57% and 73%. The reasons for the decline in the performance of treatment are mainly the creation of resistance of *H. pylori* to antibiotics and poor patient compliance with the treatment[65-67]. In Slovenia the performance of traditional 7-d treatment regimen OMC (proton pump inhibitor such as PPIs, omeprazole 2 × standard dose, metronidazole 2 × 400 mg, clarithromycin 2 × 250 mg) and OAC (PPIs, 2 × standard dose amoxicillin, 2 × 1000 mg, clarithromycin 2 × 500 mg) was last checked 10 years ago. In 1997 and 1998, the effectiveness of treatment with the scheme OMC was 82.6% and in the group treated with the scheme OAC 82%[68-70].

Globally the decline in the success of the treatment of *H. pylori* infection with a regimen that last 7 d was detected. The success of treatment is between 57% and 73%. In Europe the resistance to clarithromycin is in the range between 1% and 21.3%, metronidazole between 14.4% and 38%. In Slovenia in 2000 *H. pylori* was resistance to clarithromycin 3.7%, and to metronidazole 18.5%[71,72]. It was determined that there is still adequate resistance to clarithromycin between 15 and 20%. When a certain area exceeds this limit, it is necessary to think about replacing clarithromycin with another drug or control the sensitivity of each *H. pylori* isolate and adjust therapy to antibiogram. In 2010 in our group of Slovenian isolates we found 18.6% resistance to metronidazole and 17.5% resistance to clarithromycin. We have found that combined resistance to metronidazole and clarithromycin is 4.1%. Resistance to amoxicillin and tetracycline were not detected. Resistance to ciprofloxacin is 3.1%[73].

It is not always the fault of bacterial resistance to antibiotics for failed eradication. As mentioned above, in about 10% we fail to eradicate bacteria in the stomach, despite adequate sensitivity[72-74], and although patients followed doctor's instructions about taking antibiotics after repeated therapy failed to remove bacteria. With a better understanding of the mechanism of the immune response during infection and treatment we could explain why some patients despite antibiotic therapy do not react appropriately and the problems due to repeated infections persist and do not lead to eradication of bacteria[64,74].

Cathepsins

For this type of protease has long been thought that their application is restricted to the final degradation of proteins in lysosomes, but it was subsequently proved to be involved in several very important cellular processes. It is considered that cathepsins are involved in intra- and extracellular protein decomposition, processing propeptides and hormones, apoptosis, transformation of bone tissue, reproductive processes and the processes of differentiation, in addition, increased motility and invasion in the cells. Cysteine cathepsins are involved in various effector mechanisms of acquired and innate immune response and are essential for an effective immune response. Cathepsins are also indispensable for differentiation, adhesion and migration of immune cells, regulation of cytokines, induction of apoptosis, and many other processes[75]. Disturbed regulation of their enzymatic activity is associated with cancer, and their manipulation is shown as an option for the development of new drugs[76,77]. Cathepsins are important targets for the development of new molecules for the diagnosis, prognosis and therapy of cancer[78,79].

The activity of cathepsins is controlled by regulating the synthesis and processing of cathepsins, inhibition of endogenous inhibitors (stefins and cystatins) and pH stability[80]. Conventional cathepsins are lysosomal enzymes which are only active at acidic pH. This has changed the fact that many cathepsins in physiological and pathological conditions diverted from lysosomes into the extracellular space in other cellular organelles or in the cytoplasm and can be active at neutral pH[81].

Cathepsin X and the immune response to infection with H. pylori

Cathepsin X is a lysosomal cysteine protease located in macrophages gathered from gastric mucosa. Patients with *H. pylori* gastritis had a higher concentration of cathepsin X protein and cathepsin X mRNA levels in gastric mucosa compared to *H. pylori* negative patients[82]. Cathepsin X was also up-regulated in the gastric mucosa of patients with gastric cancer in contrast to patients without cancer[83].

We tested if the inhibition of cathepsin X influences the successful immune response to a *H. pylori* infection. We have proved the involvement of cathepsin X in the antigen presentation with TLRs. When THP-1 cells with different strains of *H. pylori* were stimulated, the addition of the inhibitor of cathepsin X resulted in a higher expression of TLR-4 on the membranes of THP-1 cells. This was especially true in clarithromycin sensitive strains of *H. pylori*. The expression of TLR-4 and TLR-2 was significantly higher when *H. pylori* stimulated DCs were cultivated together with cathepsin X inhibitor compared to the dendritic cells stimulated with *H. pylori* only[84].

The influence of higher expression of TLR-4 on the membranes of THP-1 cells on the production of cytokines IL-1b, IL-8, IL-10, and IL-6 was also tested. The concentrations were lower in the group of *H. pylori* strains that were resistant to clarithromycin. The same was seen in the THP-1 cells where we added bacteria along with the inhibitor of cathepsin X. It seems that the inhibition of cathepsin X influences the concentrations of cytokines, as well on the TLRs, that are crucial for efficient regulation of immune response to *H. pylori.* We discovered that strains that are resistant to clarithromycin are less immunogenic than clarithromycin sensitive strains and that they are capable of surviving an immune system attack for a prolonged period of time and as well develop resistance to clarithromycin that further attributes to eradication failure of *H. pylori*[84].

We have proved that resistance to clarithromycin can be a problem for the eradication since such strains seem to be less immunogenic. We assumed that the inhibition of cathepsin X to control the immune response in the cases with impossible eradication of *H. pylori* would not be beneficial. The immune response to infection would be delayed and thus could lead to persistence of bacteria and possible disease progression from atrophy, metaplasia to gastric cancer. On the other hand, when gastric cancer is already developed, inhibition of cathepsin X could be helpful since we could influence the process of cell senescence and also influence tumour cell growth.

CONCLUSION

After this review we can conclude that *H. pylori* are very successful bacteria avoiding host immune response to infection. Furthermore, not only infection itself, but also the immune response is important for the development of gastric cancer. Host cytokine gene polymorphisms represent just one component of complex interactions among host, pathogen, and environmental factors involved in gastric carcinogenesis. Only combination of *H. pylori* and host-associated risk factors do not always allow evaluation of gastric carcinoma. The disease progression from infection through atrophy to neoplastic transformation depends on other factors, including diet and different pathogenesis of *H. pylori* strains. Now we have learnt that the assessment of patients with *H. pylori* infection and its strain is very important and concluded that eradication of bacteria has essential meaning. We recommend that not only screening for *H. pylori* also the strain determination should have some diagnostic value, especially in the patients who already developed gastritis. Furthermore, for such patients assessment of disease progression (atrophic or metaplastic gastritis) could be followed by polymorphism determination. Altogether, conclusions indicate that host cytokine genotypes, host immune response to infection, as well as *H. pylori* strains could be important for greater risk for developing gastric cancer. However, we think those parameters alone could not predict the incidence and risk of the disease, only the combination could be of greater value.

REFERENCES

1 **Blaser MJ**. Helicobacter pylori and other gastric Helicobacter species. Mandell, Douglas and Bennett's Principle and Practice of Infectious Diseases, 7th ed. Mandell GL *et al* (editors). Elsevier, 2010

2 **Bauer B**, Meyer TF. The human gastric pathogen Helicobacter pylori and its association with gastric cancer and ulcer disease. *Ulcers* 2011; **2011**: 1-23 [DOI: 10.1155/2011/340157]

3 **Fox JG**, Megraud F. Helicobacter. In: Murray *et al* (editors). Manual of clinical microbiology. 9th ed., ASM Press, 2007

4 **Guruge JL**, Falk PG, Lorenz RG, Dans M, Wirth HP, Blaser MJ, Berg DE, Gordon JI. Epithelial attachment alters the outcome of Helicobacter pylori infection. *Proc Natl Acad Sci USA* 1998; **95**: 3925-3930 [PMID: 9520469 DOI: 10.1073/pnas.95.7.3925]

5 **Gubina M**, Tepeš B, Vidmar G, Ihan A, Logar J, Wraber B, Poljanec J, Bricelj I, Domanović D, Levičnik Stezinar S, Jeverica S, Kotnik V. Prevalenca protiteles proti Helicobacter pylori v Sloveniji v letu 2005. *Zdrav Vestn* 2006; **75**: 169-173

6 **Tepes B**, Kavcic B, Zaletel LK, Gubina M, Ihan A, Poljak M, Krizman I. Two- to four-year histological follow-up of gastric mucosa after Helicobacter pylori eradication. *J Pathol* 1999; **188**: 24-29 [PMID: 10398136 DOI: 10.1002/(SICI)1096-9896(199905)188: 1<24: : AID-PATH316>3.0.CO; 2-F]

7 **Goodwin CS**, Mendall MM, Northfield TC. Helicobacter pylori infection. *Lancet* 1997; **349**: 265-269 [PMID: 9014926 DOI: 10.1016/S0140-6736(96)07023-7]

8 **Basset C**, Holton J, Gatta L, Ricci C, Bernabucci V, Liuzzi G, Vaira D. Helicobacter pylori infection: anything new should we know? *Aliment Pharmacol Ther* 2004; **20** Suppl 2: 31-41 [PMID: 15335411 DOI: 10.1111/j.1365-2036.2004.02040.x]

9 **Satin B**, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, Tonello F, Kelleher D, Rappuoli R, Montecucco C, Rossi F. The neutrophil-activating protein (HP-NAP) of Helicobacter pylori is a protective antigen and a major virulence factor. *J Exp Med* 2000; **191**: 1467-1476 [PMID: 10790422 DOI: 10.1084/jem.191.9.1467]

10 **Kwok T**, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, König W, Backert S. Helicobacter exploits integrin for type IV secretion and kinase activation. *Nature* 2007; **449**: 862-866 [PMID: 17943123 DOI: 10.1038/nature06187]

11 **Reyes-Leon A**, Atherton JC, Argent RH, Puente JL, Torres J. Heterogeneity in the activity of Mexican Helicobacter pylori strains in gastric epithelial cells and its association with diversity in the cagA gene. *Infect Immun* 2007; **75**: 3445-3454 [PMID: 17438024 DOI: 10.1128/IAI.01951-06]

12 **Amieva MR**, El-Omar EM. Host-bacterial interactions in Helicobacter pylori infection. *Gastroenterology* 2008; **134**: 306-323 [PMID: 18166359 DOI: 10.1053/j.gastro.2007.11.009]

13 **Gebert B**, Fischer W, Weiss E, Hoffmann R, Haas R. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. *Science* 2003; **301**: 1099-1102 [PMID: 12934009 DOI: 10.1126/science.1086871]

14 **Rhead JL**, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC. A new Helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. *Gastroenterology* 2007; **133**: 926-936 [PMID: 17854597 DOI: 10.1053/j.gastro.2007.06.056]

15 **Blaser MJ**. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. *J Infect Dis* 1990; **161**: 626-633 [PMID: 2181029 DOI: 10.1093/infdis/161.4.626]

16 **Ermak TH**, Giannasca PJ, Nichols R, Myers GA, Nedrud J, Weltzin R, Lee CK, Kleanthous H, Monath TP. Immunization of mice with urease vaccine affords protection against Helicobacter pylori infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *J Exp Med* 1998; **188**: 2277-2288 [PMID: 9858514 DOI: 10.1084/jem.188.12.2277]

17 **Hunt RH**. The role of Helicobacter pylori in pathogenesis: the spectrum of clinical outcomes. *Scand J Gastroenterol Suppl* 1996; **220**: 3-9 [PMID: 8898429 DOI: 10.3109/00365529609094743]

18 **Perri F**, Cotugno R, Piepoli A, Merla A, Quitadamo M, Gentile A, Pilotto A, Annese V, Andriulli A. Aberrant DNA methylation in non-neoplastic gastric mucosa of H. Pylori infected patients and effect of eradication. *Am J Gastroenterol* 2007; **102**: 1361-1371 [PMID: 17509026 DOI: 10.1111/j.1572-0241.2007.01284.x]

19 **Naylor GM**, Gotoda T, Dixon M, Shimoda T, Gatta L, Owen R, Tompkins D, Axon A. Why does Japan have a high incidence of gastric cancer? Comparison of gastritis between UK and Japanese patients. *Gut* 2006; **55**: 1545-1552 [PMID: 16603635 DOI: 10.1136/gut.2005.080358]

20 **Wilson KT**, Crabtree JE. Immunology of Helicobacter pylori: insights into the failure of the immune response and perspectives on vaccine studies. *Gastroenterology* 2007; **133**: 288-308 [PMID: 17631150 DOI: 10.1053/j.gastro.2007.05.008]

21 **Go MF**, Crowe SE. Virulence and pathogenicity of Helicobacter pylori. *Gastroenterol Clin North Am* 2000; **29**: 649-670 [PMID: 11030079 DOI: 10.1016/S0889-8553(05)70136-9]

22 **Franco AT**, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA, Wroblewski LE, Piazuelo MB, Correa P, Peek RM. Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. *Cancer Res* 2008; **68**: 379-387 [PMID: 18199531 DOI: 10.1158/0008-5472.CAN-07-0824]

23 **Borody T**, Ren Z, Pang G, Clancy R. Impaired host immunity contributes to Helicobacter pylori eradication failure. *Am J Gastroenterol* 2002; **97**: 3032-3037 [PMID: 12492186 DOI: 10.1111/j.1572-0241.2002.07121.x]

24 **Kopitar AN**, Stegel V, Tepes B, Gubina M, Novaković S, Ihan A. Specific T cell responses to Helicobacter pylori predict successful eradication therapy. *J Infect* 2007; **54**: 257-261 [PMID: 16824607 DOI: 10.1016/j.jinf.2006.05.009]

25 **Pellicanò A**, Sebkova L, Monteleone G, Guarnieri G, Imeneo M, Pallone F, Luzza F. Interleukin-12 drives the Th1 signaling pathway

in Helicobacter pylori-infected human gastric mucosa. *Infect Immun* 2007; **75**: 1738-1744 [PMID: 17220306 DOI: 10.1128/IAI.01446-06]

26 **Schiff DE**, Rae J, Martin TR, Davis BH, Curnutte JT. Increased phagocyte Fc gammaRI expression and improved Fc gamma-receptor-mediated phagocytosis after in vivo recombinant human interferon-gamma treatment of normal human subjects. *Blood* 1997; **90**: 3187-3194 [PMID: 9376602]

27 **Yamauchi K**, Choi IJ, Lu H, Ogiwara H, Graham DY, Yamaoka Y. Regulation of IL-18 in Helicobacter pylori infection. *J Immunol* 2008; **180**: 1207-1216 [PMID: 18178861 DOI: 10.4049/jimmunol.180.2.1207]

28 **Ouyang W**, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008; **28**: 454-467 [PMID: 18400188 DOI: 10.1016/j.immuni.2008.03.004]

29 **O'Keeffe J**, Moran AP. Conventional, regulatory, and unconventional T cells in the immunologic response to Helicobacter pylori. *Helicobacter* 2008; **13**: 1-19 [PMID: 18205661 DOI: 10.1111/j.1523-5378.2008.00559.x]

30 **Rad R**, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C. CD25+/Foxp3+ T cells regulate gastric inflammation and Helicobacter pylori colonization in vivo. *Gastroenterology* 2006; **131**: 525-537 [PMID: 16890606 DOI: 10.1053/j.gastro.2006.05.001]

31 **Medzhitov R**, Janeway CA. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002; **296**: 298-300 [PMID: 11951031 DOI: 10.1126/science.1068883]

32 **Kaisho T**, Akira S. Toll-like receptor function and signaling. *J Allergy Clin Immunol* 2006; **117**: 979-987; quiz 988 [PMID: 16675322 DOI: 10.1016/j.jaci.2006.02.023]

33 **Kitamura H**, Morikawa H, Kamon H, Iguchi M, Hojyo S, Fukada T, Yamashita S, Kaisho T, Akira S, Murakami M, Hirano T. Toll-like receptor-mediated regulation of zinc homeostasis influences dendritic cell function. *Nat Immunol* 2006; **7**: 971-977 [PMID: 16892068 DOI: 10.1038/ni1373]

34 **Amedei A**, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G, de Bernard M. The neutrophil-activating protein of Helicobacter pylori promotes Th1 immune responses. *J Clin Invest* 2006; **116**: 1092-1101 [PMID: 16543949 DOI: 10.1172/JCI27177]

35 **Viala J**, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Mémet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL. Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nat Immunol* 2004; **5**: 1166-1174 [PMID: 15489856 DOI: 10.1038/ni1131]

36 **Algood HM**, Gallo-Romero J, Wilson KT, Peek RM, Cover TL. Host response to Helicobacter pylori infection before initiation of the adaptive immune response. *FEMS Immunol Med Microbiol* 2007; **51**: 577-586 [PMID: 17919297 DOI: 10.1111/j.1574-695X.2007.00338.x]

37 **Rad R**, Brenner L, Krug A, Voland P, Mages J, Lang R, Schwendy S, Reindl W, Dossumbekova A, Ballhorn W, Wagner H, Schmid RM, Bauer S, Prinz C. Toll-like receptor-dependent activation of antigen-presenting cells affects adaptive immunity to Helicobacter pylori. *Gastroenterology* 2007; **133**: 150-163.e3 [PMID: 17631139 DOI: 10.1053/j.gastro.2007.04.071]

38 **Ihan A**, Tepez B, Gubina M, Malovrh T, Kopitar A. Diminished interferon-gamma production in gastric mucosa T lymphocytes after H. pylori eradication in duodenal ulcer patients. *Hepatogastroenterology* 1999; **46**: 1740-1745 [PMID: 10430335]

39 **Jorge YC**, Duarte MC, Silva AE. Gastric cancer is associated with NOS2 -954G/C polymorphism and environmental factors in a Brazilian population. *BMC Gastroenterol* 2010; **10**: 64 [PMID: 20565800 DOI: 10.1186/1471-230X-10-64]

40 **Murphy G**, Thornton J, McManus R, Swan N, Ryan B, Hughes DJ, O'Morain CA, O'Sullivan M. Association of gastric disease with polymorphisms in the inflammatory-related genes IL-1B, IL-1RN, IL-10, TNF and TLR4. *Eur J Gastroenterol Hepatol* 2009; **21**: 630-635 [PMID: 19295440 DOI: 10.1097/MEG.0b013e3283140eea]

41 **Melo Barbosa HP**, Martins LC, Dos Santos SE, Demachki S, Assumpção MB, Aragão CD, de Oliveira Corvelo TC. Interleukin-1 and TNF-alpha polymorphisms and Helicobacter pylori in a Brazilian Amazon population. *World J Gastroenterol* 2009; **15**: 1465-1471 [PMID: 19322919 DOI: 10.3748/wjg.15.1465]

42 **Kamangar F**, Abnet CC, Hutchinson AA, Newschaffer CJ, Helzlsouer K, Shugart YY, Pietinen P, Dawsey SM, Albanes D, Virtamo J, Taylor PR. Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland). *Cancer Causes Control* 2006; **17**: 117-125 [PMID: 16411061 DOI: 10.1007/s10552-005-0439-7]

43 **Moorchung N**, Srivastava AN, Gupta NK, Ghoshal UC, Achyut BR, Mittal B. Cytokine gene polymorphisms and the pathology of chronic gastritis. *Singapore Med J* 2007; **48**: 447-454 [PMID: 17453104]

44 **Ito H**, Kaneko K, Makino R, Konishi K, Kurahashi T, Yamamoto T, Katagiri A, Kumekawa Y, Kubota Y, Muramoto T, Mitamura K, Imawari M. Interleukin-1beta gene in esophageal, gastric and colorectal carcinomas. *Oncol Rep* 2007; **18**: 473-481 [PMID: 17611673 DOI: 10.3892/or.18.2.473]

45 **Xue H**, Lin B, Ni P, Xu H, Huang G. Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1604-1617 [PMID: 20880168 DOI: 10.1111/j.1440-1746.2010.06428.x]

46 **Sitarz R**, de Leng WW, Polak M, Morsink FH, Bakker O, Polkowski WP, Maciejewski R, Offerhaus GJ, Milne AN. IL-1B -31T < C promoter polymorphism is associated with gastric stump cancer but not with early onset or conventional gastric cancers. *Virchows Arch* 2008; **453**: 249-255 [PMID: 18688641 DOI: 10.1007/s00428-008-0642-5]

47 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]

48 **Cauci S**, Di Santolo M, Ryckman KK, Williams SM, Banfi G. Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status. *BMC Med Genet* 2010; **11**: 29 [PMID: 20175886 DOI: 10.1186/1471-2350-11-29]

49 **Crusius JB**, Canzian F, Capellá G, Peña AS, Pera G, Sala N, Agudo A, Rico F, Del Giudice G, Palli D, Plebani M, Boeing H, Bueno-de-Mesquita HB, Carneiro F, Pala V, Save VE, Vineis P, Tumino R, Panico S, Berglund G, Manjer J, Stenling R, Hallmans G, Martínez C, Dorronsoro M, Barricarte A, Navarro C, Quirós JR, Allen N, Key TJ, Binghan S, Caldas C, Linseisen J, Kaaks R, Overvad K, Tjønneland A, Büchner FC, Peeters PH, Numans ME, Clavel-Chapelon F, Trichopoulou A, Lund E, Jenab M, Rinaldi S, Ferrari P, Riboli E, González CA. Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). *Ann Oncol* 2008; **19**: 1894-1902 [PMID: 18628242 DOI: 10.1093/annonc/mdn400]

50 **Zambon CF**, Basso D, Navaglia F, Belluco C, Falda A, Fogar P, Greco E, Gallo N, Rugge M, Di Mario F, Plebani M. Pro- and anti-inflammatory cytokines gene polymorphisms and Helicobacter pylori infection: interactions influence outcome. *Cytokine* 2005; **29**: 141-152 [PMID: 15652446 DOI: 10.1016/j.cyto.2004.10.013]

51 **Hou L**, El-Omar EM, Chen J, Grillo P, Rabkin CS, Baccarelli A, Yeager M, Chanock SJ, Zatonski W, Sobin LH, Lissowska J, Fraumeni JF, Chow WH. Polymorphisms in Th1-type cell-mediated response genes and risk of gastric cancer. *Carcinogenesis* 2007; **28**: 118-123 [PMID: 16885196 DOI: 10.1093/carcin/bgl130]

52 **El-Omar EM**, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003; **124**: 1193-1201 [PMID: 12730860 DOI: 10.1016/S0016-5085(03)00157-4]

53 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371 [PMID: 12891537 DOI: 10.1016/S0016-5085(03)00899-0]

54 **Hold GL**, Rabkin CS, Chow WH, Smith MG, Gammon MD, Risch HA, Vaughan TL, McColl KE, Lissowska J, Zatonski W, Schoenberg JB, Blot WJ, Mowat NA, Fraumeni JF, El-Omar EM. A functional polymorphism of toll-like receptor 4 gene increases risk of gastric carcinoma and its precursors. *Gastroenterology* 2007; **132**: 905-912 [PMID: 17324405 DOI: 10.1053/j.gastro.2006.12.026]

55 **Garza-Gonzalez E**, Bosques-Padilla FJ, Mendoza-Ibarra SI, Flores-Gutierrez JP, Maldonado-Garza HJ, Perez-Perez GI. Assessment of the toll-like receptor 4 Asp299Gly, Thr399Ile and interleukin-8 -251 polymorphisms in the risk for the development of distal gastric cancer. *BMC Cancer* 2007; **7**: 70 [PMID: 17462092 DOI: 10.1186/1471-2407-7-70]

56 **Hishida A**, Matsuo K, Goto Y, Mitsuda Y, Hiraki A, Naito M, Wakai K, Tajima K, Hamajima N. Toll-like receptor 4 +3725 G/C polymorphism, Helicobacter pylori seropositivity, and the risk of gastric atrophy and gastric cancer in Japanese. *Helicobacter* 2009; **14**: 47-53 [PMID: 19191896 DOI: 10.1111/j.1523-5378.2009.00659.x]

57 **Achyut BR**, Ghoshal UC, Moorchung N, Mittal B. Association of Toll-like receptor-4 (Asp299Gly and Thr399Ileu) gene polymorphisms with gastritis and precancerous lesions. *Hum Immunol* 2007; **68**: 901-907 [PMID: 18082569 DOI: 10.1016/j.humimm.2007.10.006]

58 **El-Omar EM**, Ng MT, Hold GL. Polymorphisms in Toll-like receptor genes and risk of cancer. *Oncogene* 2008; **27**: 244-252 [PMID: 18176606 DOI: 10.1038/sj.onc.1210912]

59 **Stubljar D**, Jeverica S, Jukic T, Skvarc M, Pintar T, Tepes B, Kavalar R, Stabuc B, Peterlin B, Ihan A. The influence of cytokine gene polymorphisms on the risk of developing gastric cancer in patients with Helicobacter pylori infection. *Radiol Oncol* 2014 [DOI: 10.2478/raon-2014-0041]

60 **Figueiredo CA**, Marques CR, Costa Rdos S, da Silva HB, Alcantara-Neves NM. Cytokines, cytokine gene polymorphisms and Helicobacter pylori infection: friend or foe? *World J Gastroenterol* 2014; **20**: 5235-5243 [PMID: 24833853 DOI: 10.3748/wjg.v20.i18.5235]

61 **Trejo-de la OA**, Torres J, Sánchez-Zauco N, Pérez-Rodríguez M, Camorlinga-Ponce M, Flores-Luna L, Lazcano-Ponce E, Maldonado-Bernal C. Polymorphisms in TLR9 but not in TLR5 increase the risk for duodenal ulcer and alter cytokine expression in the gastric mucosa. *Innate Immun* 2015; **21**: 706-713 [PMID: 25995217 DOI: 10.1177/1753425915587130]

62 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]

63 **Glupczynski Y**, Mégraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 820-823 [PMID: 11783701 DOI: 10.1007/s100960100611]

64 **Wong WM**, Gu Q, Wang WH, Fung FM, Berg DE, Lai KC, Xia HH, Hu WH, Chan CK, Chan AO, Yuen MF, Hui CK, Lam SK, Wong BC. Effects of primary metronidazole and clarithromycin resistance to Helicobacter pylori on omeprazole, metronidazole, and clarithromycin triple-therapy regimen in a region with high rates of metronidazole resistance. *Clin Infect Dis* 2003; **37**: 882-889 [PMID: 13130398 DOI: 10.1086/377206]

65 **Tepeš B**, Križman I. Priporočila za zdravljenje okužbe s Helicobacterjem pylori v Sloveniji. *Zdrav Vestn* 1998; **67**: 159-162

66 **Bochenek WJ**, Peters S, Fraga PD, Wang W, Mack ME, Osato MS, El-Zimaity HM, Davis KD, Graham DY. Eradication of Helicobacter pylori by 7-day triple-therapy regimens combining pantoprazole with clarithromycin, metronidazole, or amoxicillin in patients with peptic ulcer disease: results of two double-blind, randomized studies. *Helicobacter* 2003; **8**: 626-642 [PMID: 14632678 DOI: 10.1111/j.1523-5378.2003.00179.x]

67 **Tepeš B**, Gubina M. Razlogi za neuspeh antimikrobnega zdravljenja okužbe z bakterijo Helicobacter pylori in naše terapevtske možnosti. *Zdrav Vestn* 2004; **73**: 503-506

68 **Tepeš B**. Primerjava dveh trotirnih antimikrobnih shem zdravljenja okužbe z bakterijo Helicobacter pylori. *Zdrav Vestn* 2000; **69**: 505-508

69 **Laine L**, Hunt R, El-Zimaity H, Nguyen B, Osato M, Spénard J. Bismuth-based quadruple therapy using a single capsule of bismuth biskalcitrate, metronidazole, and tetracycline given with omeprazole versus omeprazole, amoxicillin, and clarithromycin for eradication of Helicobacter pylori in duodenal ulcer patients: a prospective, randomized, multicenter, North American trial. *Am J Gastroenterol* 2003; **98**: 562-567 [PMID: 12650788 DOI: 10.1016/S0002-9270(02)06010-0]

70 **Rokkas T**, Sechopoulos P, Robotis I, Margantinis G, Pistiolas D. Cumulative H. pylori eradication rates in clinical practice by adopting first and second-line regimens proposed by the Maastricht III consensus and a third-line empirical regimen. *Am J Gastroenterol* 2009; **104**: 21-25 [PMID: 19098844 DOI: 10.1038/ajg.2008.87]

71 **Vakil N**, Megraud F. Eradication therapy for Helicobacter pylori. *Gastroenterology* 2007; **133**: 985-1001 [PMID: 17854602 DOI: 10.1053/j.gastro.2007.07.008]

72 **Jeverica S**, Tepeš B, Ihan A, Skvarč M. Primarna odpornost bakterije Helicobacter pylori. *Zdrav Vestn* 2010; **97**: 25-30

73 **Ihan A**, Tepez B, Kavcic I, Gubina M. Il-2 receptor expression on gastric mucosa T lymphocytes is enhanced in duodenal ulcer patients compared with non-ulcer dyspeptic patients. *Hepatogastroenterology* 1996; **43**: 1665-1670 [PMID: 8975986]

74 **Colbert JD**, Matthews SP, Miller G, Watts C. Diverse regulatory roles for lysosomal proteases in the immune response. *Eur J Immunol* 2009; **39**: 2955-2965 [PMID: 19637232 DOI: 10.1002/eji.200939650]

75 **Mohamed MM**, Sloane BF. Cysteine cathepsins: multifunctional enzymes in cancer. *Nat Rev Cancer* 2006; **6**: 764-775 [PMID: 16990854 DOI: 10.1038/nrc1949]

76 **Zavasnik-Bergant T**, Turk B. Cysteine proteases: destruction ability versus immunomodulation capacity in immune cells. *Biol Chem* 2007; **388**: 1141-1149 [PMID: 17976006 DOI: 10.1515/BC.2007.144]

77 **Turk B**, Turk D, Turk V. Lysosomal cysteine proteases: more than scavengers. *Biochim Biophys Acta* 2000; **1477**: 98-111 [PMID: 10708852 DOI: 10.1016/S0167-4838(99)00263-0]

78 **Premzl A**, Zavasnik-Bergant V, Turk V, Kos J. Intracellular and extracellular cathepsin B facilitate invasion of MCF-10A neoT cells through reconstituted extracellular matrix in vitro. *Exp Cell Res* 2003; **283**: 206-214 [PMID: 12581740 DOI: 10.1016/S0014-4827(02)00055-1]

79 **Grabowskal U**, Chambers TJ, Shiroo M. Recent developments in cathepsin K inhibitor design. *Curr Opin Drug Discov Devel* 2005; **8**: 619-630 [PMID: 16159024]

80 **Goulet B**, Baruch A, Moon NS, Poirier M, Sansregret LL, Erickson A, Bogyo M, Nepveu A. A cathepsin L isoform that is devoid of a signal peptide localizes to the nucleus in S phase and processes the CDP/Cux transcription factor. *Mol Cell* 2004; **14**: 207-219 [PMID: 15099520 DOI: 10.1016/S1097-2765(04)00209-6]

81 **Bühling F**, Peitz U, Krüger S, Küster D, Vieth M, Gebert I, Roessner A, Weber E, Malfertheiner P, Wex T. Cathepsins K, L, B, X and W are differentially expressed in normal and chronically inflamed gastric mucosa. *Biol Chem* 2004; **385**: 439-445 [PMID: 15196006 DOI: 10.1515/BC.2004.051]

82 **Krueger S**, Kuester D, Bernhardt A, Wex T, Roessner A. Regulation of cathepsin X overexpression in H. pylori-infected gastric epithelial cells and macrophages. *J Pathol* 2009; **217**: 581-588 [PMID: 19090485 DOI: 10.1002/path.2485]

83 **Krueger S**, Kalinski T, Hundertmark T, Wex T, Küster D, Peitz U, Ebert M, Nägler DK, Kellner U, Malfertheiner P, Naumann

M, Röcken C, Roessner A. Up-regulation of cathepsin X in Helicobacter pylori gastritis and gastric cancer. *J Pathol* 2005; **207**: 32-42 [PMID: 16025436 DOI: 10.1002/path.1820]

84 **Skvarc M**, Stubljar D, Kopitar AN, Jeverica S, Tepes B, Kos J, Ihan A. Inhibition of cathepsin X enzyme influences the immune response of THP-1 cells and dendritic cells infected with Helicobacter pylori. *Radiol Oncol* 2013; **47**: 258-265 [PMID: 24133391 DOI: 10.2478/raon-2013-0043]

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