JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2006, 57, Supp 3, 15–27
www.jpp.krakow.pl

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INTERPLAY BETWEEN *HELICOBACTER PYLORI* AND THE IMMUNE SYSTEM. CLINICAL IMPLICATIONS

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Helicobacter pylori (*H. pylori*) is a gram-negative bacteria infecting more than 50% of human population. *H. pylori* selectively colonizes gastric mucosa and represents the major cause of gastroduodenal pathologies, such as gastric ulcer, autoimmune *gastritis*, gastric cancer and B cell *lymphoma* of mucosa associated lymphoid tissue (MALT). In this review interplay between *H. pylori* and both innate and adaptive immune responses is discussed. The second part of this article presents current knowledge about the relationship between *H. pylori* infection and neoplasia.

Key words: *Lymphoid tissue, autoimmune gastritis, MALT lymphoma, gastric ulcer cancer*

INTRODUCTION

Our bodies are constantly exposed to different microorganisms that are present in the environment. However, contact with pathogenic microorganisms rarely results in infection. This is because our bodies are protected by both innate and adaptive immune mechanisms.

The innate immune system consists of many cells such as: macrophages, dendritic cells, mast cells, neutrophils, eosinophils and NK cells. The cells of the innate immune system become activated during inflammation, which is virtually always a sign of infection with pathogenic microbes (1). The main goal of these cells is to get rid of the infection. It is worthy to underline that innate responses depend on host recognition of highly conserved structures called “pathogen-associated molecular patterns” (PAMPs), present in microorganisms (2). PAMPs are recognized by structures known as “pathogen recognition receptors” (PRR)

(2). The two major currently recognized groups of such receptors in humans are toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-containing proteins (3). Whereas TLRs are associated with the plasma membrane or, in some case, with lysosomal and/or endosomal vesicles, both NOD1 and NOD2 are expressed in the cytosol (4).

However, in certain types of infection, the innate immune system is not able to deal with the infection and then adaptive immune response is required. In such infections, the innate immune system can instruct the adaptive immune system about the nature of the pathogen *via* expression of CD80 and CD86 co-stimulatory molecules on dendritic cells and creation of cytokine milieu.

There are two major classes of adaptive immune responses. The first class of adaptive immune response is mediated by MHC II restricted antigen-specific T-cell receptor (TCR) $\alpha\beta^+$ $CD4^+$ (delayed-type hypersensitivity, DTH) that belong to the population of Th1 cells or by MHC I restricted $CD8^+$ (T-cell-mediated cytotoxicity) lymphocytes and are induced by intracellular pathogens (5). In effector phase of DTH, the Th1 lymphocytes release proinflammatory cytokines like IFN-γ which induce local tissue cells to produce chemokines that recruit and activate an infiltrate of bone marrow-derived leukocytes (6). CD8 T cytotoxic (Tc) cells kill infected host cells *via* released perforin and granzymes and by triggering FasL dependent apoptosis.

The second type of adaptive immune response is the humoral immune response mediated by antibodies produced by B lymphocytes (1). In this type of immune response B cells receive support from Th2 lymphocytes that release IL-4, IL-5, IL-6 and IL-13. The main function of the humoral response is to destroy extracellular microorganisms and prevent the spread of infection. For complete health every living being must be continuously protected from infection and tumors by their immune system. At the same time there must be some mechanisms protecting the organism from development of inappropriate immune responses that are harmful to ones own body (allergy, autoimmunity) and those that help to silent the inflammatory responses and allow their resolution. This points to the importance of the balance of the immune response and its strict control by regulatory mechanisms.

It is commonly accepted that self-tolerance is based on two major mechanisms: clonal deletion in the thymus and anergy in the periphery (7, 8). However, at present there is a strong evidence that these two mechanisms responsible for self-tolerance are additionally supported by the action of T suppressor (Ts) cells also called T regulatory (Treg) cells.

At present it is well accepted that regulatory cells that inhibit immune response belong to a big family of cells that include different T cell populations. First population of Treg cells belongs to naturally occurring $CD4^+$ $CD25^+$ lymphocytes that develop directly from $CD4^+$ T cell precursors during positive selection as a result of their interaction with thymic epithelial cells (9). Second group of $CD4^+$ Treg cells known as induced Tregs comprise Tr1 and Th3 cell

populations (10). Last two groups of T cells with suppressor activity belong to $CD8^+$ and NKT cell populations (11).

Pathogenesis of H. pylori

Helicobacter pylori (*H. pylori*) is transmitted from person to person by the oral-oral and fecal-oral route (12). *H. pylori* infection is usually acquired in childhood and may persist for life of the patient (13). As described in previous articles of this issue, the infection rates vary in different parts of the world with average rates of 40-50% in western countries and 80-90% in Asia and Far East countries. Most (about 80%) of the infected humans are designated as asymptomatic, whereas in 10-15% of patients, *H. pylori* infection leads to peptic ulcer, *gastritis*, cancer or gastric lymphoma of mucosal-associated lymphoid tissue (MALT) (14-16). Immunopathology of *H. pylori* is summarized in *Fig. 1.*

The following factors play the crucial role in *H. pylori* pathogenicity: cag-associated pathogenicity island (*cag* PAI), vacuolation cytotoxin (VacA), CagA

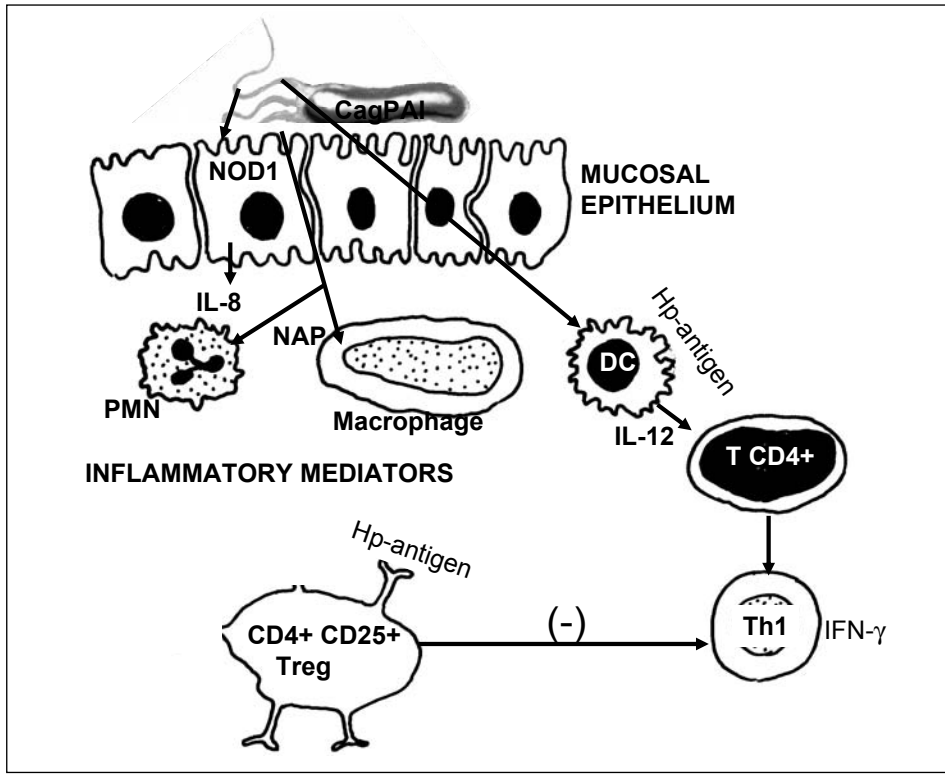

Fig. 1. Interplay between *H. pylori* and the immune system. *H. pylori* induces both innate (macrophage, neutrophil and dendritic cell (DC) activation) and adaptive immune responses. Th1 effector cells of adaptive immunity are negatively regulated by T regulatory (Treg) cells.

protein and neutrophil-activating protein (NAP). The *cag* PAI encodes proteins that are thought to mediate functions analogous to those of type IV secretion system present in certain bacteria. The *H. pylori cag* PAI mediates the translocation of the effector protein, CagA into gastric epithelial cells (17). VacA causes massive vacuolar degradation of epithelial cells *in vitro* and epithelial erosions *in vivo* (18). It was recently demonstrated that CagA is involved in disruption of the apical-epithelial junction (19) whereas, NAP plays an important role in recruitment and activation of inflammatory cells in the lamina propria (20).

It is worthy to underline that *H. pylori* has achieved a balance in which the immune system is stimulated sufficiently to cause inflammation and epithelial cell damage at the site of infection, perhaps as means to survive in the nutrient-poor environment between the epithelium and the mucus layer, while modulating the response to prevent elimination of the bacteria (21). The interaction between *H. pylori* and host immune system will be discussed in further paragraphs of the review.

THE ROLE OF INNATE IMMUNE SYSTEM IN H. PYLORI INFECTION

Influence of H. pylori on gastric epithelial cells

When microorganism invades the human body, the first barrier they meet and have to cross to establish an infection are epithelial cells lining the gastrointestinal, urogenital or respiratory tract. Epithelial cells of the gastric mucosa are the first cells that recognize *H. pylori* that infect stomach through the glicolipid recepts located at the surface of epithelial cells and adhesions produced by the *bacterium*. It is commonly accepted that PRR, like TLRs and NOD1 and NOD2, belong to microbial sensors in innate immunity. There are some reports showing that gastric epithelial cells are equipped to express TLR 2, TLR4, TLR5 and TLR9 (22). However, as shown by others gastric epithelial cells do not express TLRs and it is in line with their lack of response to *H. pylori* LPS or flagellin that are TLR 4 and TLR5 ligands, respectively. Another PRR, NOD1 is expressed in gastric epithelial cells and seems to be involved in initiation of inflammatory response during *H. pylori* infection. Indeed, it was shown that cagPAI-positive bacteria induce NF-κB and synthesis of neutrophil-attracting chemokine IL-8 (CXCL8) in gastric epithelial cells (23). This finding is supported by experiments employing NOD1-deficient mice that have impaired control of *H. pylori* densities in the stomach (23).

In summary, presented information suggests that recognition of *H. pylori* by some of PRR e.g. NOD1 can cause release of IL-8 and other proinflammatory cytokines by gastric epithelial cells what subsequently initiates inflammatory response.

Neutrophils, macrophages and dendritic cells and their response to H. pylori

As mentioned above gastric epithelial cells in response to *H. pylori* produce IL-8, which is a very potent neutrophil chemotactic factor (23). Additionally,

NAP a cytosolic protein is released by bacterial lysis and interacts directly with neutrophils and monocytes to activate their inflammatory function (24). NAP activates both neutrophils and monocytes to produce reactive oxygen species (ROS) by activating the plasma membrane NADPH. On the other hand, *H. pylori* to neutralize negative effects of ROS produces enzymes e.g. catalase and superoxide dismutase involved in ROS scavenging (25). *H. pylori* also activates inducible nitric oxide synthase (iNOS) and production of bactericidal agent such as nitric oxide (NO) in macrophages (26). It is worthy to mention that NO produced in large quantities, apart from its antimicrobial activity, may also lead to gastric epithelial cell injury (27) and apoptosis (28) what contributes to *H. pylori* pathogenesis. It is important to know that *H. pylori* that induces synthesis of NO can also inhibit its production by synthesis of an arginase that compete with iNOS for their substrate (21). This mechanism can protect *H. pylori* from deleterious effects of NO. It may be suggested that *H. pylori* maintains a delicate balance between activating inflammatory responses and protecting itself from the negative consequences (21). This balance between inflammation and its inhibition may perhaps contribute to *H. pylori* survival in the nutrient-poor environment between the epithelium and the mucus layer (21). It is commonly known that macrophages are equipped with TLRs to sense microbes. However, many studies showed that peritoneal macrophages from TLR2-/-, TLT4-/- or Myd88-/- mice produce similar levels of IL-6 in response to *H. pylori* as those observed in wild type mice (29). These data are in keeping with the finding that *H. pylori* LPS is about 1000 times less effective than *E. coli* LPS in inducing proinflammatory cytokine production in macrophages (30). It is suggested that other factors than TLR ligands e.g. *H. pylori* HSP60 may participate in macrophage activation during *H. pylori* infection (29).

Dendritic cells represent an important population of antigen presenting cells that can be found in the gastrointestinal mucosa. These cells express various TLRs that recognize many structures on pathogenic microbes. However, it is interesting that rapid maturation of dendritic cells in response to intact *H. pylori* is independent of *H. pylori* LPS (31). It is suggested that dendritic cell activation and IL-12 production might be the result of *cag*PAI expression and stimulation (32). Subsequently IL-12 producing dendritic cells become able to influence the development of adaptive immunity towards Th1 mediated response.

Adaptive immune response to H. pylori

In many experiments it was shown that protection from *H. pylori* infection is mediated by T lymphocytes, whereas humoral response does not seem to be essential in anti *H. pylori* response because protection could be induced in antibody-deficient mice (33). Studies in MHC class I-deficient mice have shown that protective immunity could be induced but not in animals lacking MHC II (34, 35). These findings suggest that CD4 T lymphocytes are involved in anti *H. pylori*

immune response, whereas MHC class I restricted CD8 T cells are not essential. Further experiments showed the presence of IL-12 (36) and IL-18 (37) in the gastric mucosa. Both of these cytokines are responsible for directing gastric T lymphocytes to Th1 mediated response. Experimental data in animal model showing that *H. pylori* preferentially triggers Th1 response are supported by clinical observations. In peptic ulcer RT-PCR analysis of antral biopsies showed IL-12, IFN-γ and TNF-α but not IL-4, mRNA expression (38). The antigen specificity experiments showed that CagA is the immuno-dominant antigen of *H. pylori* specific for T cell responses in the stomach of peptic ulcer patients (39). It should be mentioned that preferential Th1 mediated response to *H. pylori* infection is not fully protective, as infection may persist for life. Moreover, gastric Th1 lymphocytes can damage the epithelium directly or indirectly by producing cytokines that induce inflammation (40). Predominant activation of Th1 lymphocytes that release IFN-γ and TNF-α, in the absence of Th2 cytokines, can increase release of gastrin that stimulates H^+ and pepsinogen secretion from oxyntic glands (39, 41). The hypothesis that Th1 cells play an important role in the pathogenesis of peptic ulcer is supported by observations showing that suppression of Th1 response or activation of Th2 cells result in amelioration of clinical symptoms. It was shown that treatment of mice with IFN-γ induced *gastritis* with enhanced levels of gastrin and reduced levels of somatostatin, whereas Th2 releases cytokine IL-4, suppressing the release of gastrin, through a mechanism that required release of somatostatin from D cells, and reduced colonization with *H. pylori* in chronically infected mice (19, 42). Additionally, it was found that another Th2 cytokine IL-10 reduces the degree of *gastritis* induced by *H. pylori* (43). Summing up, it was shown that elevated levels of mRNA for interleukin-12p40 (IL-12p40), gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), and inducible nitric oxide synthase (iNOS) were associated with gastroprotection in mice immunized with *H. pylori*, but Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) and chemokines (KC, MIP-2, and MCP-1) expression was not associated with such protection (44). Therefore, it may be assumed that never-ending Th1-driven inflammation would result in immunopathology e.g. autoimmune *gastritis* (45) whereas, a polarized Th2 response would not be beneficial for the host because it does not provoke the protection (39). Only an efficient *H. pylori* – specific Th1 response appropriately tuned by Th2 cells would lead to protection.

Recent studies show that T cells involved in protection from *H. pylori* are under control of CD4+ CD25+ Treg cells. It has been shown in humans that *H. pylori* infection impairs *H. pylori*-specific memory CD4+ T cells through antigen-specific CD4+ CD25+ Treg cells and it might be involved in the persistence of infection (46). It is also speculated that Tregs in asymptomatic individuals keep the pathology mild enough to avoid symptoms. Indeed, it was shown that removal of Tregs from the memory T cell population increased the proliferative responses to *H. pylori* antigens and importantly, addition of Tregs back to the memory T cells suppressed the *H. pylori* specific response (47). Further work has shown that

CD4+ CD25+ Treg cells isolated from the gastric and duodenal mucosa of *H. pylori* infected asymptomatic patients express the specific Treg marker FOXP3, suggesting an important role for Tregs in maintaining a balance between chronicity and development of symptoms at the site of infection (47, 48). In summary, protective function of Tregs against severe *gastritis* in *H. pylori* infection is not achieved without costs. Recently, it was shown that the protective effect of Tregs against *gastritis* was associated with more extensive bacterial colonization (48). Interplay between *H. pylori* and the immune system is presented in *Fig. 2.*

The relationship between H. pylori infection and neoplasia

Humans infected with *H. pylori* develop *gastritis* that can persist for decades. A biological consequence of long-term infection accompanied by inflammation is increased risk of developing of gastric cancer (49). It should be stressed, however,

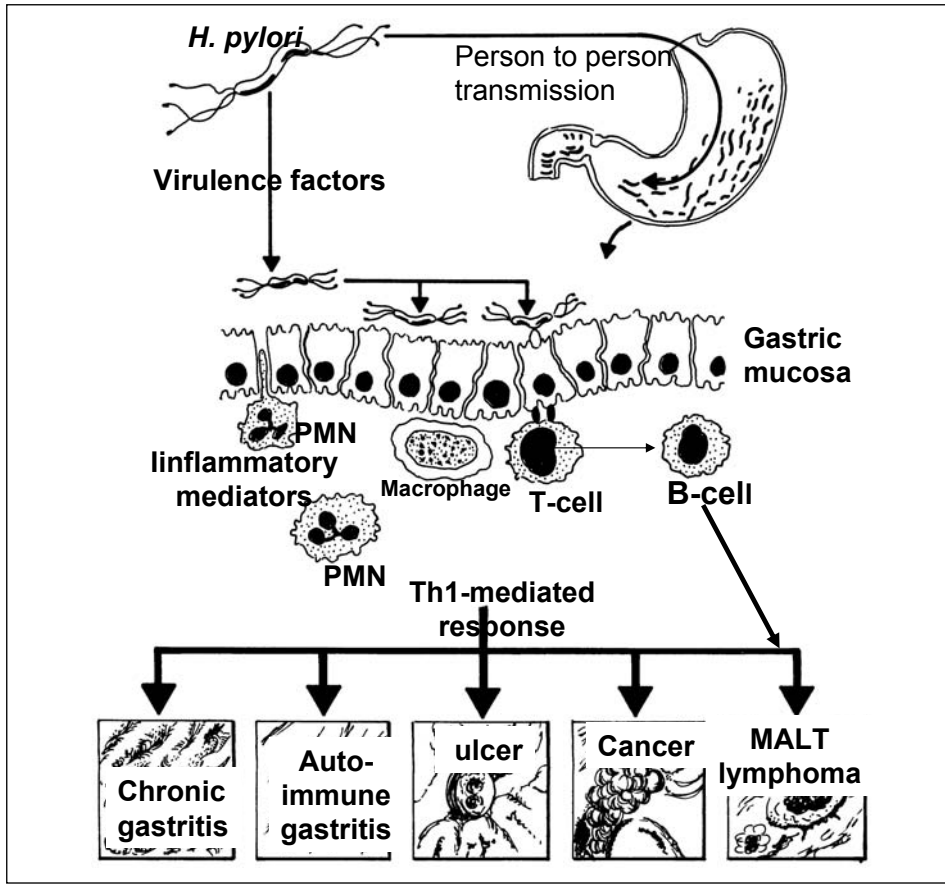

Fig. 2. Immunopathology of *H. pylori* infection. Infection with *H. pylori* may result in: acute/chronic *gastritis*, autoimmune *gastritis*, peptic ulcer, gastric cancer or MALT lymphoma.

that only a small percentage (1-2%) of *H. pylori* infected humans ever develop neoplasia, and show an increased cancer risk involves specific interactions between pathogen and host, which, in turn, are dependent on strain-specific bacterial factors and inflammatory responses governed by host genetic diversity (50). It was already shown that all *H. pylori* strains cause *gastritis* but only CagA+ expressing strains augment the risk of severe *gastritis*, atrophic *gastritis* and gastric cancer (51). It is suggested that increased risk of developing *adenocarcinoma* in patents infected with cag+ strains is related to their strong ability to induce IL-8 production by gastric epithelial cells and subsequent neutrophil infiltration of gastric mucosa and development of inflammatory response (52).

More recently, it was shown in animal model that there is a relationship between host immune response and the pathogenesis of gastric cancer. It was found that immune deficient mice such as RAG2-/- or SCID that do not possess either T or B lymphocytes develop high levels of colonization when infected with *H. pylori*, whereas, very little epithelial damage or preneoplastic changes have been found (53). Further experiments showed that B cell deficient mice develop severe atrophy and metaplasia, whereas T cell deficient mice are protected from changes induced by *H. pylori* infection, suggesting a role for T cells in disease initiation and progression (54). Experiments employing different strains of mice showed that animals prone to develop Th1 immunity after *H. pylori* inoculation are more susceptible to atrophy and metaplasia, whereas mice that have prominent Th2 polarized response are resistant to atrophy and metaplasia (55). These data suggest that cytokine imbalance might be responsible for disease progression and may decide about the disease outcome after *H. pylori* infection. Accordingly, factors involved in regulating cytokines may confer susceptibility to or protection against *H. pylori*-associated diseases (56). It was observed that a single nucleotide polymorphism in the coding of promoter regions of cytokine or cytokine receptor may affect cytokine synthesis, causing either high or low production of a given cytokine. Therefore, variant cytokine alleles might contribute to individual differences in inflammatory responses and account for heterogenous outcomes after infection (56). Such a correlation was shown in gastric *carcinoma* in which IL-1β polymorphism confer a 2 fold increased risk for gastric *adenocarcinoma* after *H. pylori* infection (57). Polymorphism in other cytokine genes also increase the risk of gastric *carcinoma* after *H. pylori* infection. Proinflammatory polymorphism in the promotor region of TNF-α, (58), IL8 (59) and polymorphism linked to decreased synthesis of anti-inflammatory cytokine IL-10 (60) are risk factors for the development of gastric cancer. At present it is accepted that an increased risk for gastric *carcinoma* with carriage of multiple cytokine polymorphisms in IL-1β, IL1RN, IL-10 and TNF-α in the context of *H. pylori* infection is associated with a proinflammatory phenotype and therefore an increased risk for DNA damage and, ultimately, gastric *carcinoma* (50, 56, 61).

Another neoplastic disease strongly linked with *H. pylori* infection is MALT *lymphoma*. This assumption was made on the basis of observation that *H. pylori* infection significantly increased the risk for gastric MALT *lymphoma* because the vast majority of gastric MALT *lymphoma* patients were infected with *H. pylori* (62). Moreover, eradication of *H. pylori* with antibiotics alone resulted in regression of gastric MALT *lymphoma* in 75% cases (63) and most of the patients showed long-term clinical remission (64). Gastric MALT *lymphoma* results from the uncontrolled polyclonal expansion of a subset of memory B cells. The B cells of MALT *lymphoma* share phenotype of marginal zone B cells ($CD20^+$, $CD21^+$, $CD35^+$, IgM^+ and IgD^-) (65). MALT lymphoma B cells proliferate in response to CD40 costimulation and cytokines produced by *H. pylori* activated T helper cells (66). On the other hand, the surface immunoglobulin on gastric MALT lymphoma B cells does not recognize *H. pylori*, but instead recognize various autoantigens, suggesting that malignant cells are transformed from autoreactive B lymphocytes (56, 67). Further work showed that both Th1 and Th2 type cytokines are necessary for the development of MALT *lymphoma* (68). *In vitro* studies showed that *H. pylori* stimulation of T cells induced *H. pylori*-specific Th clones derived from gastric MALT *lymphoma* to express strong help for B cell activation and

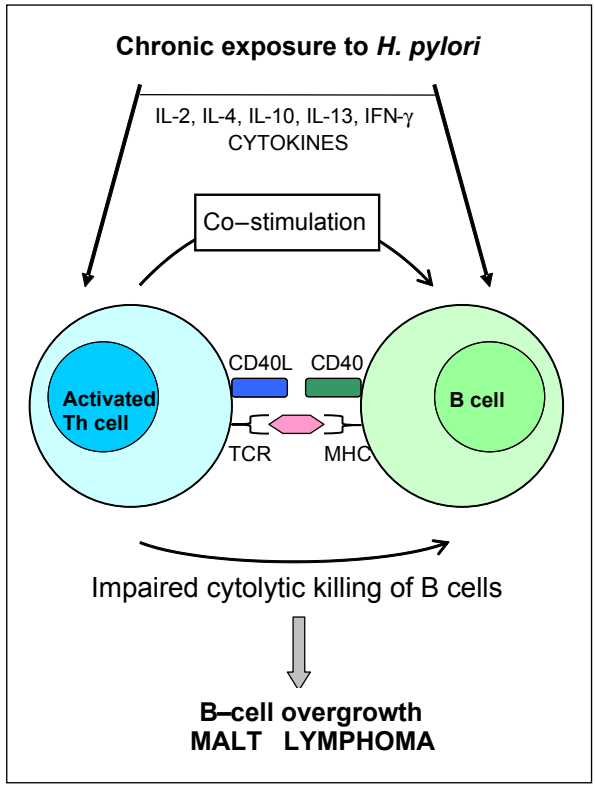

Fig. 3. Factors involved in the development of MALT *lymphoma*. Chronic production of cytokines and delivery of costimulatory signals by Th cells together with impaired cytolytic killing of B cells results in B cell overgrowth and the development of MALT lymphoma.

proliferation. In contrast, in patients with *gastritis* without MALT *lymphoma* the helper function of gastric T cells was negatively regulated by the concomitant cytolytic killing of B cells (69). The mechanism of MALT *lymphoma* development is summarized in *Fig. 3*. None of the T cell clones isolated from MALT *lymphoma* patients was able to express perforin-mediated cytotoxicity against autologous B cells and Fas-FasL mediated apoptosis in target cells was defective as well (70). The reason why gastric T cells from MALT *lymphoma* possess strong helper activity and are defective in mechanisms negatively controlling B cell growth still remains unclear.

Acknowledgements: This work was supported by grants from the Polish Committee of Scientific research (KBN, Warsaw) No. 3 PO 5B 091 25, 2 PO 5A 157 28 and 2PO 5A 208 29.

The author is indebted to Dr. W. Ptak for his advice and encouragement.

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