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THE ROLE OF DEFENSINS IN DEVELOPMENT OF CHRONIC STOMACH MUCOSITIS IN CHILDREN WITH HELICOBACTER INFECTION

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Contemporary concepts of pathogenic role of antimicrobial peptides — defensins — in children with Helicobacter pylori are given here. The material is based on corresponding literature and own observations.
Key words: Helicobacter pylori, mucostitis, children.

Abbreviations: **AMP** — antimicrobial peptides; **AP-1** — a transcription factor the activator protein 1; **CagA** — cytotoxin associated gene A; **CXCL** — α chemokine; **EGFR** — the epidermal growth factor receptor; **HBD** — hemoglobin subunit delta; **HD** — human defensin of Paneth cells; **HNP** — Human Neutrophil Peptide; **IL** — interleukin; **IRF** — interferon regulating transcription factor; **JNK** — c-Jun N-terminal kinase; **LPS** — lipopolysaccharide; **MAPK** — mitogen-activated protein kinases; **MD-2** — factor 2 of myeloid differentiation; **NF-kB** — nuclear factor kappa-light-chain-enhancer of activated B cells; **NLR** — NOD-likely receptors; **PAMP** — pathogen-associated molecular patterns; **SNP** — single-nucleotide polymorphism; **TLR** — Toll-likely receptors; **TNF** — tumor necrosis factor.

The discovery of *Helicobacter pylori (H. pylori)* by R. Warren and B. Marshall, Australian scientists, in 1983 dramatically changed the scientific view about pathogenesis of inflammatory diseases of gastrointestinal tract.

The researchers Barry James Marshall and Robin Warren singled out and cultivated spiral microorganisms found on stomach mucous membrane in a patient suffering from gastritis. In 1985, the described microorganism appeared in international taxonomy for bacteria under the name of *Campylobacter pyloridis*. In 1987, *Campylobacter pyloridis* was renamed *Campylobacter pylory*, and having finally proved that this causative agent didn't belong to the *Campylobacter* genus, the bacterium was identified as *H. pylori*. The term «*Helicobacter*» reflects two morphological features of the causative agent: *helical* — spiral and *bacter* — rod-like [8].

Helicobacter infection is one of the most widely spread chronic human infections. In different parts of the world, the quantity of infected with *H. pylori* adults varies 40 to 90%, and in CIS-countries the figure is 70–80%. *H. pylori is* clinically significant pathogen which is responsible for considerable part of common sickness rate and death rate in the whole world. In 1994 the conciliation commission of US National Institute of Health admitted that *H. pylori* had a key role in development of ulcer. And International Agency for Research on Cancer (France) defined *H. pylori* as Type I carcinogen [4,7].

Early childhood is a critical period when the risk to be infected with *H. pylori* is especially high. The probability to be infected with *H. pylori* decreases with the age. The most probable way of infection transmission is from mother to child and from child to child. The risk to be infected sharply correlates in the case of infected mother or own brothers/sisters [37,14]. Today, *H. pylori* is the only known microorganism which is able to ecize in aggressive acidic medium of human stomach. *H. pylori* is an especial microorganism which has been infecting people for millenniums, it has a specific place of existence — *H. pylori* adhesion takes place only on stomach epithelium, on areas of stomach metaplasia, on duodenum or on heterotopia of duodenum [12].

Forming of protein molecules with strong antimicrobial activity is one of the oldest unspecific mechanisms of macrooranism's defense against infectious pathogens' invasion. Antimicrobial peptides (AMP) are the most effective and impressive molecular components of unspecific defense mechanisms.

Antimicrobial peptides are small amphiphilic (that is, having hydrophilic and hydrophobic parts) molecules consisting of only 12–50 amino-acid residues and having impressive bactericidal effect. In evolutional respect, defensins, earlier known as lysosomal cationic proteins, are the oldest proteins taking part in unspecific defense of microorganism against infectious agents. Continuously increasing interest in biological and medical significance of defensin peptides and high level

Table

Macroorganism's identification receptors and corresponding PAMP *H. pylori* [25]

Identification receptors	PAMP *H. pylori*
TLR 1/2/6	Membrane protein HpaA
TLR 4	tetra-LPS (lightly immune-genic), hexa-LPS
TLR 5	Flagelin (very light immunogen)
TLR 9	Bacterial nucleic acids
NLRC 4	?
NLRC 1	peptidoglycan

of their gens' mutability, let to consider them molecules surviving renascence [26]. For the first time, alpha defensins were singled out in a separate structural-functional group in 1980. The group included neutrophil or myeloid alpha defensins (HNP-1, HNP-2, HNP-3, HNP-4), and intestinal alpha defensins of Paneth cells (HD5, HD6). Neutrophil alpha defensins take part in antibacterial defense, and alpha-defensins being secreted by Paneth cells prevail in regulating of microflora vital activity in intestine [17,43].

The subclass of β-defensins (HBD) unites over 40 peptides which are key components of unspecific antiinfectious defense system of mucous membrane of respiratory, gastrointestinal urogenital tracts and cutaneous covering. There are four best studied species of β-defensins: HBD-1, HBD-2, HBD-3 and HBD-4 [30]. Various signaling pathways take part in synthesis regulation and release of defensins in children with helicobacter infection.

Agnes Katalin Kocsis and coauthors established, that SNP rs1799946 (G-52A) of *DEFB1* gen is credibly connected with chronic gastritis associated with *H. pylori* [9,41].

Hajime Isomoto and coauthors established that concentration of HNP-1, HNP-2 of HNP-3 in gastric juice of patients suffering from chronic gastritis associated with *H. pylori* is considerably higher than in non-infected patients [16]. Content of neutrophil defensins in blood serum does not depend on contamination with *H. pylori*, while concentration of HNP-1, HNP-2 and HNP-3 in gastric juice correlates with IL-8/CXCL8 content and content of neutrophils in infected areas of stomach mucous membrane. High content of myeloid alpha defensins is detected in tissue samplings of stomach mucous membrane of children and adults infected with *H. pylori* [44].

Contamination with *H. pylori* is accompanied with growth of мРНК HBD expression and with forming of HBD peptides by epithelial cells of stomach epithelium [3,24,40]. Concentration of HBD-2 in gastric juice is higher in patients suffering from chronic gastritis associated with *H. pylori* [13]. In epithelial cell lines of stomach mucous membrane pronounced HBD-2 and HBD-4 expression is induced only by *H. pylori* strains, whose genome contains cytotoxic-associated gene CagA in PAI — pathogenicity island. Pathogenicity island of *H. pylori* genome contains over 40 gens defining pathogenicity of bacterium. The gen encodes immunodominant protein CagA which is a marker of strains' high malignancy [1,15,19,36]. Presence of T4SS gens in Cag PAI is invariable attribute of malignancy. These gens encode macromolecular structures which function as tiny needles for transmission of bacterial products from *H. pylori* to the host's cells modulating metabolism of epithelial cells in stomach mucous membrane [5]. At the same time, мРНК and HBD-1 expression by epithelial cell lines of stomach mucous membrane, which is registered in 24 hours after contamination with *H. pylori*, does not depend on the presence of CagA gen [41]. HBD-2 induction is not a unique *H. pylori's* response to effect of protein CagA. Other bacteria, such as salmonella also activate HBD-2 expression by human stomach epithelial cells. But it has not been still defined if induction of HBD-2 expression is directly connected with causative agent or it just indicates that active inflammatory process is present, because expression degree of this peptide correlates with activity degree of inflammatory reaction of stomach mucous membrane [15,20].

Membrane, endosome TLR, as well as cytoplasmic NLR sensors, take part in induction of defensins expression, associated by *H. pylori* (see table) [1,2,19].

Epithelial cells of stomach mucous membrane express membrane-bound TLR2, TLR4, TLR5 and endosomal TLR9. During some time, the role of TLR-4-associated disorder in development of inflammation, in the case of contamination with helicobacter infection, was excluded, because TLR4 was not identified on epithelial cells of antrum during aggravation of disease [23].

But Leisa Mandell and coauthors [29] proved that cytokine-inducing effect of LPS *H. pylori* is mediated by TLR4. Macrophages without TLR4 stay intact after stimulation with LPS *H. pylori*. It is established that

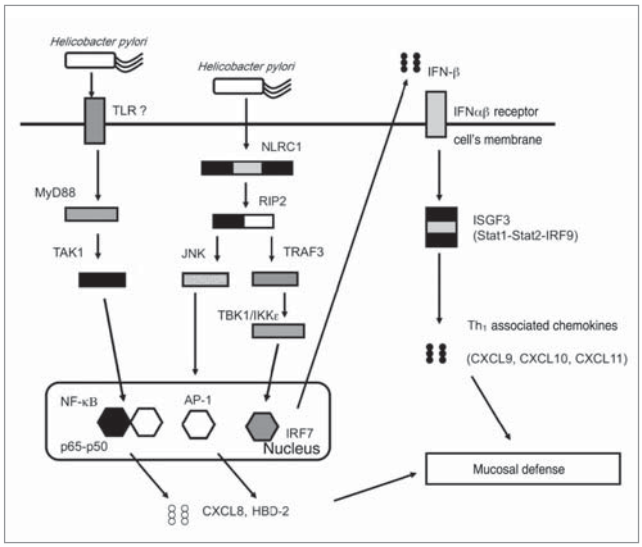

Fig. Molecular mechanisms of antihelicobacter mucosal defense (T. Watanabe and coauthors, 2011 with additions)

activation of TLR4, situated on apical surface of epithelial cells' membrane of stomach mucous membrane, is caused by interaction of LPS *H. pylori* hexa-forms. During development of helicobacter infection, the expression of TLR4 and MD 2 by epithelial cells grows, while TLR2 and TLR5 expression practically does not change, though they take part in development of inflammation associated by helicobacter infection [33].

In our previous researches we showed that aggravation of disease in children suffering from chronic gastroduodenitis, associated by *H. pylori*, is accompanied with expression rising of key LPS-sensor in stomach mucous membrane which is TLR4-receptor. Clinical course of chronic gastroduodenitis, not associated by *H. pylori*, unlikely to chronic gastroduodenitis, associated by H. pylori, is accompanied with considerable growth of accessory molecule sCD14. Probably, low concentration of sCD14 assists *H. pylori's* invasion [2].

The role of TLR4-associated disorder in development of inflammatory process depends on concentration of sCD14. When sCD14 concentration is considerably high its interaction with LPS *H. pylori* complex and LPS-binding protein causes transmission of LPS to HDL (high-density lipoprotein) with further excretion of this exotoxin from the microorganism. Thus, sCD14 decreases expressing of LPS-associated effects. When sCD14 concentration is low, LPS *H. pylori* interacts with mCD14 which further leads to forming of LPS/TLR4/MD-2 complex and to excitation of proinflammatory intracellular tracts. It is interesting that induction of cytokine response to LPS-influence of all clinically significant helicobacter bacteria is probably associated also with TLR2 [29]. The growth of TLR4 and TLR2 expression by macrophages and dendritic cells of own plate in stomach mucous membrane is recorded. Excitation signal, associated with TLR, activates NF-κB, AP-1 and MAPK transcription factors that causes induction of expression and secretion of IL-1, IL-6, IL-8/CXCL8, IL-18 and TNF-α proinflammatory cytokines and HBD-2 defensin [21,10,39]. It is possibly that forming of HBD-2 is induced also by intracellular signal pathways associated with TLR5 and TLR9, though according to most researches these receptors cannot play important role in development of helicobacter-associated inflammation of stomach mucous membrane [35,25].

It is shown that muramyldipeptide *H. pylori* activates intracellular NLRC1 receptor which excites RIP2 → TRAF3 → TBK1 → IKK → IRF7 signal way activating synthesis of I-type interferons and JNK → AP-1 signal cascade inducing β-defensins (fig.) [6,32,45].

Pargeet K. Boughan with coauthors demonstrated that *H. pylori* stimulates HBD-2 and HBD-3 secretion using different method of cell recognition: HBD-2 expression is induced when a disorder is caused by derivatives of helicobacter peptidoglycan of NLRC1 receptor, while HBD-3 expression is induced after activation of EGFR-associated signal receptor. HBD2 expression is the intrinsic quality of CagA contamination with positive *H. pylori* strains, and HBD3 expression does not depend on the presence of a product of CagA gen. Taking into account that disorder of NLRC1 and TLR-2 receptors causes formation of IL-8/CXCL-8, it is possible to assume that there are both direct and indirect mechanisms of activation of HBD-2 associated with PAMP *H. pylori* effect. It means that induction of HBD-2 synthesis can be either directly connected with activation of NF-κB transcription factor by NLRC1 and TLR2-associated signal cascades, or indirectly — with action of IL-8/CXCL-8 [38]. CagA protein can intensify TLR2-associated excitation signal, as it is shown that Cag-A positive *H. pylori* induce more active production of IL-8/CXCL-8 than CagA negative *H. pylori* TLR-2 and CD14 expressing HEK 293 by cells [29].

Excitation by PAMP *H. pylori* of TLR and NLR epithelial cells and antigen-presenting cells of immune system leads to development of Th1- and Th_{17} — associated response in stomach mucous membrane playing decisive role in ablation of causative agent of disease [6]. In patients with chronic inflammatory disease of gastroduodenal zone associated with *H. pylori* high level of IL-17 is observed in mucous membrane tissues of antrum which is caused by activity of TH_{17}-cells. It is known that HBD-2 peptide recruits TH_{17}-cells when interacting with CCR 6-receptor

[11]. It is still unknown how much β-defensins contribute to Th17 response, associated with *H. pylori*, causing not only ablation, but also including production of IL-8/CXCL8, and involving of neutrophils to the area of damaged mucous membrane which increases the risk of ulceration [31].

Expression degree of HBD-3 in epithelial cell lines of stomach mucous membrane, infected with *H. pylori*, is 11,5 times higher than in uninfected cells. Synthetic defensins HBD-2 and HBD-3 show bactericidal activity in concentrations 30 mg/ml [20] and 50 mg/ml correspondingly [24,28]. Chronic gastritis associated with *H. pylori* is associated with forming of separate Paneth cells in metaplasial areas of mucous membrane which express HD5 and HD6 [27, 22].

The role of defensins in *H. pylori* ablation has not been definitely established. Some authors believe that concentration and expression levels of defensins in patients with chronic gastritis are not enough for ablation of *H. pylori* [34]. There is also assumption that *H. pylori* strains causing chronic inflammation of stomach mucous membrane are resistant to AMP [42]. But Alexandra Grubman and coauthors demonstrated direct involvement of HBD-2 in effective killing of *H. pylori* [45].

Ozlem Bekem Soylu and coauthors count that further researches are required to establish the role of defensins in ablation of *H. pylori* [44].

Thus, antimicrobial peptides, in particular defensins which directly damage pathogens, are molecular effectors of unspecific inborn defense mechanisms of macroorganism against pathogenic infectious agents including *H. pylori*. The use of antimicrobial and immunomodulatory features of defensins for treatment of children suffering from chronic inflammatory gastroduodenal diseases, especially in the time of sharp growth of *H. pylori's* antibiotic resistance, reveals potentially new therapeutic opportunities as to effectiveness of eradication therapy. Taking this into account, the search of optimal methods of defensin response modulation in children with helicobacter infection may be the object of further investigations.

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