

# Different effectiveness of *Helicobacter pylori* lipopolysaccharides with or without Lewis<sup>XY</sup> determinants in stimulating the secretion of proinflammatory cytokines IL-8 and TNF- $\alpha$ by peripheral blood mononuclear leukocytes

Odmierna skuteczność lipopolisacharydów *Helicobacter pylori* różniących się ekspresją determinantów antygenowych Lewis<sup>XY</sup> w pobudzaniu leukocytów jednojądrzastych krwi obwodowej do wydzielania cytokin prozapalnych: IL-8 i TNF- $\alpha$

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**Słowa kluczowe:** *Helicobacter pylori*, lipopolisacharydy, interleukina 8, czynnik martwicy nowotworów  $\alpha$ .

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## Abstract

**Introduction:** *Helicobacter pylori* is an aetiological agent of chronic gastritis, gastric and duodenal ulcers, and gastric cancers. It is suggested that *H. pylori* must have undergone evolutionary changes that enable the bacteria to overcome the host immune response. The molecular mimicry between Lewis (Le) determinants present in *H. pylori* lipopolysaccharide (LPS) and on the host cells may play a role in the outcome of *H. pylori* infections.

**Aim:** In this study we investigated the production of inflammatory cytokines tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 8 (IL-8), by human peripheral blood mononuclear leukocytes (PBML) from *H. pylori* infected (11) and uninfected (10) subjects (21 women, aged 25–50 years), in response to *H. pylori*-LPS with Le<sup>XY</sup>(+) or without Le<sup>XY</sup>(-) determinants.

**Material and methods:** Peripheral blood was collected using the Vacutainer heparin system and constituted a source of total or monocyte and lymphocyte enriched PBML fractions. Tumour necrosis factor  $\alpha$  and IL-8 were assessed by immunosorbent assay (ELISA) in the supernatants from

## Streszczenie

**Wstęp:** Pałeczki *Helicobacter pylori* są przyczyną przewlekłego zapalenia błony śluzowej żołądka, owrzodzeń żołądka lub dwunastnicy oraz nowotworów żołądka. Prawdopodobnie bakterie te podlegały takim zmianom ewolucyjnym, które umożliwiły im przystosowanie się do życia w organizmie gospodarza poprzez unikanie jego mechanizmów obronnych. Podobieństwo molekularne pomiędzy determinantami antygenowymi Lewis (Le) występującymi w lipopolisacharydzie (LPS) *H. pylori* oraz na komórkach gospodarza może odgrywać ważną rolę w procesach przystosowawczych tych bakterii.

**Cel:** W pracy oceniono wytwarzanie cytokin prozapalnych: czynnika martwicy nowotworów  $\alpha$  (TNF- $\alpha$ ) oraz interleukiny 8 (IL-8) przez leukocyty jednojądrzaste krwi obwodowej dawców (21 kobiet w wieku 25–50 lat), zakażonych (11) lub niezakażonych (10) *H. pylori*, w odpowiedzi na LPS *H. pylori* Le<sup>XY</sup>(+) lub Le<sup>XY</sup>(-).

**Materiał i metody:** Leukocyty krwi obwodowej otrzymane z wykorzystaniem heparynizowanego systemu Vacutainer stanowiły źródło pełnej zawiesiny leukocytów jednojądrza-

leukocyte cultures stimulated or not with *H. pylori* LPS or standard *Escherichia coli* LPS.

**Results:** The results showed that adherent but not non-adherent PBML responded to *H. pylori*-LPS by TNF- $\alpha$  and IL-8 production. The *H. pylori*-LPS LeXY(+) was a stronger stimulus for macrophage-derived cytokines, as compared with *H. pylori*-LPS LeXY(-). *Helicobacter pylori*-LPS LeXY(+) driven production of TNF- $\alpha$  and IL-8 was significantly inhibited with antibodies to LPS-binding protein (LBP) and to CD14 proteins.

**Conclusions:** It is possible that LeXY determinants of *H. pylori*-LPS could be involved in LPS signalling for inflammatory cytokines and thus regulate the outcome of *H. pylori* infections.

## Introduction

*Helicobacter pylori* has been recognized as a major causative agent of chronic gastritis, gastric and duodenal ulcers and gastric cancer [1]. However, its ability to cause chronic persistent colonization, growth and active adaptation to the acidic environment of the human stomach and resistance to the host defence mechanisms is still under discussion [2]. It is suggested that *H. pylori* bacteria must have undergone evolutionary changes that enable them to survive the host immune response. On the other hand, they must express highly diverse virulence factors contributing to the disease development [3, 4]. However, not only bacterial but also host factors such as genetic predisposition play an important role in the development, maintenance and the outcome of the disease. Several functional polymorphisms have been described in the genes encoding cytokines and their receptors [5, 6]. Experimental animal models have confirmed that the host response is an important determinant in the severity of gastritis [7]. Two systems of immune response, innate and acquired, are involved in the eradication of infectious agents. Innate immunity provides a rapid, unspecific host defence but is also necessary for the development of an acquired, specific response that should overcome the infection. In the early stages of infection, *H. pylori* lipopolysaccharide (LPS) activates the innate immune system and induces secretion of the chemotactic factors such as interleukin 8 (IL-8), as well as production of other proinflammatory cytokines including IL-1, IL-6 and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) [8, 9]. These molecules are responsible for recruitment of inflammatory cells: neutrophils, monocytes, macrophages, mast cells, as well as lymphocytes T and B.

It is suggested that *H. pylori* may use mechanisms similar to other pathogens or commensals to achieve

stych (*peripheral blood mononuclear leukocytes* – PBML) oraz zawiesin wzbogaconych w monocyty lub limfocyty. Stężenie TNF- $\alpha$  i IL-8 w supernatantach z hodowli niestymulowanych lub stymulowanych LPS *H. pylori* LeXY(+) lub LeXY(-) oraz wzorcowym LPS *Escherichia coli* oceniano w immunoenzymatycznym teście ELISA.

**Wyniki:** Głównym źródłem TNF- $\alpha$  i IL-8, w odpowiedzi na LPS *H. pylori*, była frakcja PBML wzbogacona w monocyty. Wykazano, że LPS *H. pylori* LeXY(+) silniej stymulował wytwarzanie obu cytokin pochodzenia makrofagowego niż LPS typu LeXY(-). Sekrecja TNF- $\alpha$  i IL-8 przez PBML stymulowane LPS *H. pylori* LeXY(+) była istotnie hamowana w środowisku przeciwciał przeciwko białkom LBP (białko wiążące LPS) i CD14.

**Wnioski:** Można przypuszczać, że determinanty antygenowe LeXY występujące w LPS *H. pylori* mogą modulować ścieżki sygnałowe cytokin prozapalnych i na tej drodze regulować przebieg takich zakażeń.

the suppression or evasion of the innate immune system [10, 11]. *Helicobacter pylori* has developed the ability to vary its LPS chemistry and thereby subvert the recognition by innate immune receptors [12, 13]. The majority of *H. pylori* strains express in their LPS the Lewis (Le) determinants (LeX, LeY, Le<sup>a</sup>, Le<sup>b</sup>) that are similar to the epitopes present on the gastric epithelial cells of the host [14]. *Helicobacter pylori* may modulate the expression of Lewis antigens in response to variable environmental conditions in the stomach. This may facilitate persistent colonization [15]. The increased adherence of *H. pylori* to the gastric mucosa enhances the interaction between the bacterial and the host cells, which is followed by activation of the nuclear factor  $\kappa$ B (NF- $\kappa$ B) transcription factor and the signal transduction pathway [16]. The phase-variable expression of Lewis antigens allows the bacteria to modulate the host adaptive immune response through interactions with DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin) on dendritic cells and macrophage subpopulations [17]. Although it is known that macrophages are the main target of bacterial LPS, the interaction between *H. pylori* LPS (differing in the expression of Lewis determinants) and cells of the monocyte/macrophage lineage is poorly understood. We focused attention on a potential impact of such cells in the production of cytokines playing a crucial role in initiation of the immune response towards infectious agents.

## Aim

The aim of this study was to evaluate the secretory activity of PBML isolated from *H. pylori* infected and uninfected donors, concerning IL-8 and TNF- $\alpha$  production, in response to *H. pylori* LPS of LeXY(-) or LeXY(+) type. The non-separated PBML as well as monocyte or

lymphocyte enriched PBML fractions were used as targets for *H. pylori* LPS. The monoclonal anti-CD14 and anti-LBP antibodies were used for assessment of the CD14-dependent signalling pathway for IL-8 and TNF- $\alpha$  secretion in response to *H. pylori* LPS.

## Material and methods

### Subjects

Twenty-one healthy women (25-50 years old) participated in this study and signed their consent. The Local Ethical Committee approved the study protocol. The *H. pylori* status was assessed by  $^{13}\text{C}$  urea breath test (UBT) [18] and anti-*H. pylori* antibody production detected in ELISA assay as previously described [19]. The volunteers were divided into two groups, one with UBT and anti-*H. pylori* IgG positive – *H. pylori* infected – H.p. (+) and the other with UBT and anti-*H. pylori* IgG negative – *H. pylori* non-infected – H.p. (–). They did not receive any antibiotics, bismuth salts, non-steroidal anti-inflammatory or immunosuppressive agents.

### Leukocyte cultures

Peripheral blood was collected using the Vacutainer system with heparin as an anticoagulant factor. In the experiments, the samples of peripheral blood (30 ml) from 21 donors, 11 *H. pylori* positive H.p. (+) and 10 *H. pylori* negative H.p. (–) were separated by Lymphoprep-gradient centrifugation as recommended by the manufacturer (Nycomed Pharma AS, Norway) in order to obtain PBML. The total PBML population ( $5 \times 10^5$  in 100  $\mu\text{l}$ /well) in 96-well microplates was stimulated for 24 h (37°C, 5%  $\text{CO}_2$ ) in triplicate with 100  $\mu\text{l}$ /well of standard *E. coli* LPS (Sigma, St. Louis, Michigan, US) or with *H. pylori* LPS, with or without Lewis XY determinants (200 ng/ml). Spontaneous cytokine activity of the cells was assessed in the cultures in a complete medium without any stimulus. The experiments were performed in the RPMI-1640 medium containing 10% fetal calf serum (FCS), 200 mM L-glutamine and 50  $\mu\text{g}/\text{ml}$  gentamicin (complete medium). The *H. pylori* LPS was prepared by the hot phenol-water procedure after pre-treatment of bacterial biomass with protease. Then the LPS preparation was purified by RNase, DNase and proteinase K treatment and by ultracentrifugation, as previously described [12]. In some experiments the cell suspensions enriched with monocytes or lymphocytes were used. For this purpose, the total fraction of mononuclear leukocytes was incubated for 1 h (37°C, 5%  $\text{CO}_2$ ) in culture plates, to obtain the adherent and non-adherent cell fractions. The non-adherent cells were used for preparing the lymphocyte-enriched fraction,

whereas the remaining adherent cells were a source of monocyte-enriched fraction. The role of CD14 receptor in cytokine signalling and secretion, in response to *H. pylori* LPS, was estimated using the cells pre-treated for 15 min (37°C, 5%  $\text{CO}_2$ ), with mouse monoclonal antibodies against human CD14 and lipopolysaccharide binding protein (LBP) molecules (HyCult Biotechnology, Uden, The Netherlands) in a concentration of 10  $\mu\text{g}/\text{ml}$  before stimulation with *H. pylori* LPS preparations.

After 24-h incubation of the cells, the cell culture supernatants were collected in order to estimate IL-8 and TNF- $\alpha$  concentration, centrifuged and frozen at –20°C in 100  $\mu\text{l}$  aliquots.

### Assessment of cytokine secretion

The cell culture supernatants were assayed to determine the level of IL-8 and TNF- $\alpha$ . The cytokine levels in the cell culture supernatants were measured by ELISA method using commercial assays (Qantikine® Human TNF- $\alpha$ , Immunoassay and Qantikine® Human IL-8 Immunoassay (R&D System, Minneapolis, USA), as recommended by the manufacturer.

In all experiments, the wells coated with anti-human IL-8 or anti-human TNF- $\alpha$  monoclonal mouse antibody were filled with the reagents for the control: known concentrations of recombinant human IL-8 (rhIL-8) or rhTNF- $\alpha$ , anti-human IL-8 or with anti-human TNF- $\alpha$  polyclonal goat IgG antibody labelled with horseradish peroxidase (HRP), and with a chromogenic substrate, OPD, for development of a colour reaction. The absorbance values were measured in a Victor 2 counter (Wallac Oy, Turku, Finland) at  $\lambda = 450$  nm and  $\lambda = 490$  nm. Based on the absorbance values (A) for each rhIL-8 and rhTNF- $\alpha$  dilution, the standard curve was constructed on the basis of the equation for a straight line:  $y = ax + b$ . The parameters “a” and “b” were calculated from the slope and the y-intercept, respectively, of the drawn line. The concentrations of IL-8 and TNF- $\alpha$  in the culture supernatants were estimated on the basis of the following formula: IL-8 (ng/ml) =  $A - b/a$  or TNF- $\alpha$  (pg/ml) =  $A - b/a$  (A – absorbance, a – the regression coefficient, b – the point of the line’s intersection with the Y-axis).

### Statistical analysis

Statistica 5.5 PL software with non-parametric tests was used: Mann-Whitney U test (for unpaired data) to verify the hypothesis that the two compared samples came from two statistically different populations; and  $\chi^2$  test to compare the prevalence of the analysed parameters in the studied groups.

## Results

### TNF- $\alpha$ and IL-8 production by PBML from *H. pylori* infected and *H. pylori* uninfected individuals

Production of TNF- $\alpha$  and IL-8 is pivotal in initiation of the immune response to pathogens. The contribution of TNF- $\alpha$  to improvement of the adhesive molecules' expression on the surface of endothelial cells and the role of IL-8 as a chemotactic factor for immunocompetent cells are well established. In this study, the levels of TNF- $\alpha$  and IL-8 were estimated in culture supernatants of PBML from *H. pylori* infected or uninfected donors, stimulated with *H. pylori* LPS with or without Lewis XY determinants, and with the referent *E. coli* LPS. The levels of TNF- $\alpha$  and IL-8 were estimated using the ELISA test. The sensitivity of the test was determined based on the analysis of the mean value of absorbance for controls and dilutions of the TNF- $\alpha$  and IL-8 standards. All of the PBML from *H. pylori* (+) and *H. pylori* (-) volunteers produced TNF- $\alpha$  and IL-8 spontaneously as well as in response to *H. pylori* LPS with or without Lewis XY determinants, and to standard *E. coli* LPS.

The intensity of the TNF- $\alpha$  secretion varied between individuals. However, differences in the mean cytokine quantities were observed (Figure 1 A). In both groups of donors the TNF- $\alpha$  production by PBML in response to *H. pylori* LPS of LeXY(-) type was very low, even slightly lower than in the milieu of culture medium alone. By comparison, the TNF- $\alpha$  production by PBML in response to *H. pylori* LPS of LeXY(+) type as well as to *E. coli* LPS was significantly higher as compared to the natural secretion level and to the response to *H. pylori* LPS of LeXY(-) type ( $p < 0.05$ ). However, there were no significant differences between the groups of *H. pylori* (+) and *H. pylori* (-), in the mean production of TNF- $\alpha$  by PBML stimulated with *H. pylori* LPS of Lewis XY(+) or Lewis XY(-) type, the LPS of *E. coli* or those unstimulated.

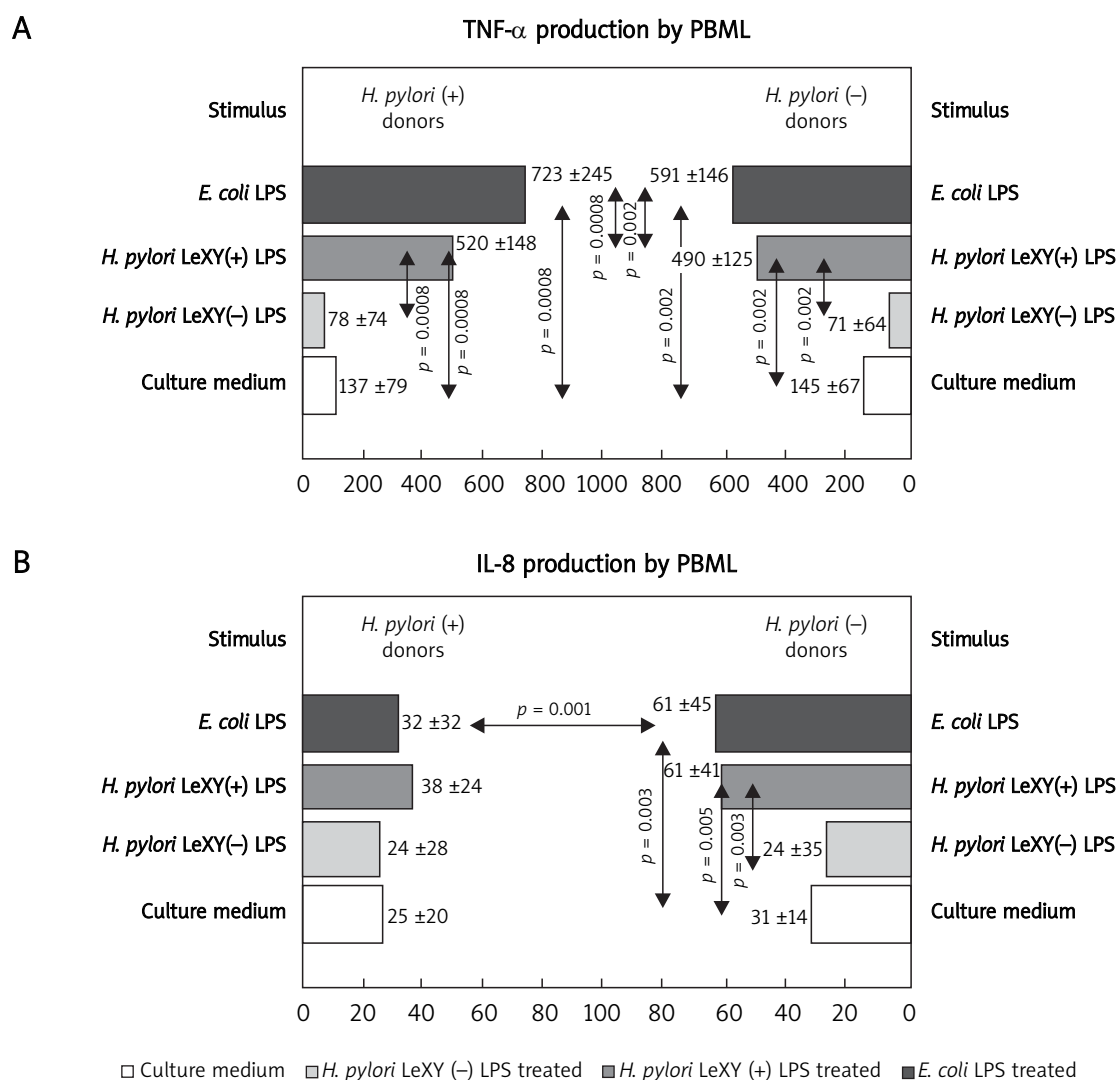
Figure 1 B shows the secretion of IL-8 by PBML, unstimulated or stimulated with *H. pylori* or *E. coli* LPS, from the volunteers negative or positive for *H. pylori* infection. Similarly, the intensity of the secretion of IL-8 varied between the individuals. Among *H. pylori* (+) donors the production of IL-8 by PBML unstimulated or stimulated with *H. pylori* LPS of LeXY(-) or LeXY(+) type as well as by *E. coli* LPS did not differ significantly. However, in the group of *H. pylori* (-) donors the mean secretion of IL-8 was significantly higher in response to *H. pylori* LPS of LeXY(+) type and to *E. coli* LPS, as compared to natural IL-8 production, and to the secretion of such cytokine in response to *H. pylori* LPS of LeXY(-) type. A comparative analysis of IL-8 secretion by PBML unstimulated or stimulated with *H. pylori* and *E. coli* LPS in the

group of *H. pylori* infected or uninfected donors was performed. The mean intensity of IL-8 production by PBML from *H. pylori* (-) donors was higher than the production of such cytokine by leukocytes from *H. pylori* (+) individuals when *H. pylori* LPS of LeXY(+) type and *E. coli* LPS were used for PBML stimulation. In the case of *E. coli* LPS the difference was statistically significant (Figure 1 B).

In this study the *H. pylori* LPS of LeXY(+) type stimulated the PBML, in the group of *H. pylori* infected or uninfected donors, more effectively than *H. pylori* LPS of LeXY(-) type. To determine which leukocyte fraction produced TNF- $\alpha$  and IL-8 in response to *H. pylori* LPS of LeXY(+) type, the PBML from both groups of donors, *H. pylori* (+) and *H. pylori* (-) (which responded by production of both cytokines), were separated in order to prepare the monocyte or lymphocyte enriched cell suspensions. A clear distinction between monocytes and lymphocytes as regards the TNF- $\alpha$  and IL-8 production in response to *H. pylori* LPS of LeXY(+) type was observed. The monocytes consisted of the main leukocyte fraction responding by TNF- $\alpha$  and IL-8 production when stimulated with *H. pylori* LPS of LeXY(+) type (Figure 2).

### Inhibition with monoclonal anti-CD14 and anti-LBP antibodies of TNF- $\alpha$ and IL-8 production by PBML from *H. pylori* infected or uninfected subjects, in response to *H. pylori* LPS

In order to know whether *H. pylori* LPS signalling for the production of two inflammatory cytokines, IL-8 and TNF- $\alpha$ , involves the cell surface and soluble molecules, CD14 and LBP, respectively, the PBML cultures in the presence or absence of monoclonal anti-CD14 and anti-LBP antibodies were used for stimulation of the cells with *H. pylori* LPS of LeXY(+) type. Figure 3 shows the mean intensity of TNF- $\alpha$  (Figure 3 A) and IL-8 (Figure 3 B) production in PBML cultures from three *H. pylori* (+) (D1-D3) and two *H. pylori* (-) (D4, D5) donors, with or without anti-CD14 and anti-LBP antibodies, in response to *H. pylori* LPS of LeXY(+) type. In all cases the TNF- $\alpha$  production was totally abolished in the milieu of anti-CD14 and anti-LBP antibodies. In two cases (donors D2, D3) the anti-CD14 antibody significantly decreased the production of TNF- $\alpha$  by PBML (Figure 3 A). The IL-8 production by PBML cultures from four out of five donors was completely stopped with the anti-CD14 antibody (donors D2-D5) and with the anti-LBP antibody (donors D1, D2, D4, D5). Significant inhibition of IL-8 secretion was detected for PBML cultures from donor D1 after treatment of the cells with the anti-CD14 antibody, and for PBML cultures from donor D3 after pre-treatment of the cells with the anti-LBP antibody (Figure 3 B).



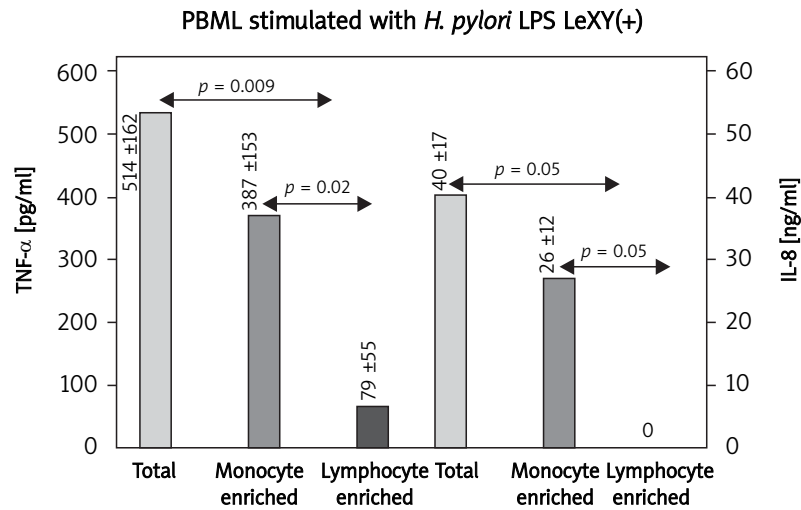
**Fig. 1.** Comparison of TNF- $\alpha$  (A) and IL-8 (B) production by peripheral blood mononuclear leukocytes (PBML) from *H. pylori* infected (*H. pylori* (+)) and *H. pylori* uninfected (*H. pylori* (-)) donors, unstimulated (culture medium) or stimulated with *H. pylori* lipopolysaccharide (LPS) with (*H. pylori* LPS LeXY(+)) or without (*H. pylori* LPS LeXY(-)) Lewis determinants or with standard *E. coli* LPS

**Ryc. 1.** Porównanie wydzielania TNF- $\alpha$  (A) oraz IL-8 (B) przez leukocyty krwi obwodowej (PBML) dawców zakażonych (*H. pylori* (+)) lub niezakażonych (*H. pylori* (-)) pączkami *H. pylori*, niestymulowane (podłoże hodowlane) lub stymulowane LPS *H. pylori* z determinantami antygenowymi Lewis (*H. pylori* LPS LeXY(+)) lub bez (*H. pylori* LPS LeXY(-))

### Discussion

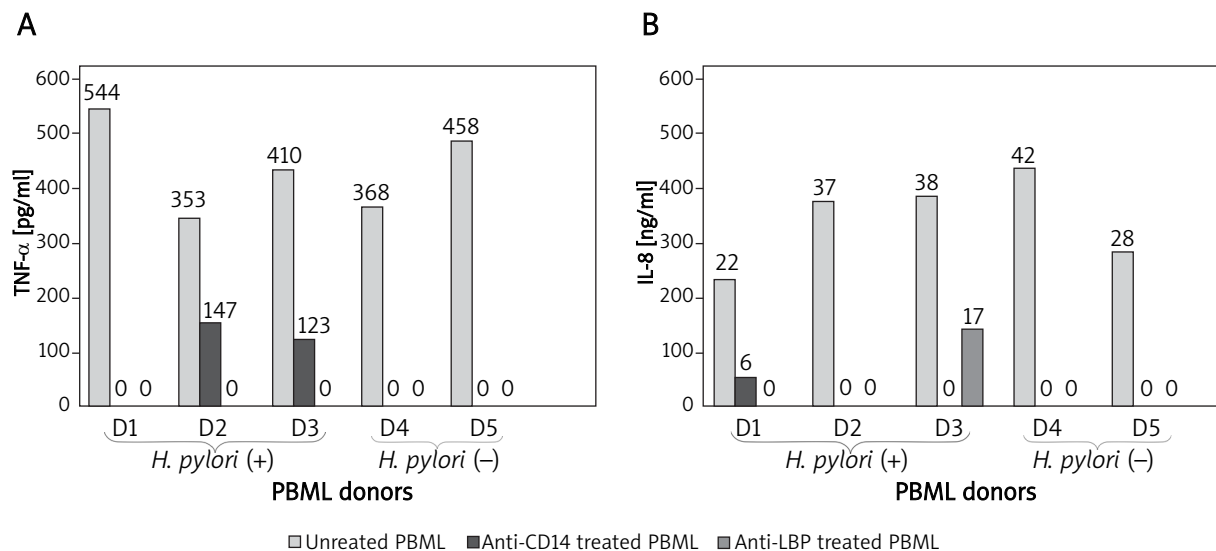
The majority of *H. pylori*-infected individuals are asymptomatic despite persistent chronic infection. The reason why some individuals remain *H. pylori*-infected for life but without any symptoms while others develop severe diseases is unknown, but presumably the discrepancy results from multifactorial interactions among host immunological and physiological factors, bacterial virulence determinants and several environmental influ-

ences that modulate the host response. The balance of bacterial factors and host inflammatory response to *H. pylori* infection determines the disease outcome. Even in asymptomatic subjects, *H. pylori* induces histological gastritis, which is characterized by a dense infiltration of granulocytes, monocytes and lymphocytes in gastric mucosa. The production of inflammatory cytokines plays a primary role in *H. pylori*-induced gastroduodenal diseases. Interleukin-8 has been shown to



**Fig. 2.** Comparison of TNF- $\alpha$  and IL-8 production by non-separated peripheral blood mononuclear leukocytes (PBML) from selected donors, as well as by monocyte or lymphocyte enriched PBML fractions, in response to *H. pylori* lipopolysaccharide (LPS) of Lewis XY type [LeXY(+)]

**Ryc. 2.** Porównanie wytwarzania TNF- $\alpha$  i IL-8 przez komórki jednojądrzaste krwi obwodowej wybranych dawców, nierozdzielone lub wzbogacone w monocyty lub limfocyty, w odpowiedzi na lipopolisacharyd (LPS) *H. pylori* mający determinanty antygenowe Lewis XY [LeXY(+)]



**Fig. 3.** Inhibition of TNF- $\alpha$  (A) and IL-8 (B) production by peripheral blood mononuclear leukocytes (PBML) from selected donors, untreated or pre-incubated with monoclonal anti-CD14 and anti-LBP (LPS binding protein) antibodies in response to LPS with Lewis XY determinants [LeXY(+)]

**Ryc. 3.** Zahamowanie wytwarzania TNF- $\alpha$  (A) i IL-8 (B) przez leukocyty jednojądrzaste krwi obwodowej (PBML) wybranych dawców, nietraktowane lub traktowane przeciwciałami monoklonalnymi anty-LBP (białko wiążące lipopolisacharyd – LPS) lub anty-CD14, w odpowiedzi na LPS *H. pylori* mający determinanty antygenowe Lewis XY [LeXY(+)]

play a key role in this event. Direct contact of whole bacteria with epithelial cells might be critical for IL-8 induction *in vivo* [20]. The ability of *H. pylori* strains to induce IL-8 secretion from *H. pylori*-activated gastric epithelial cells and neutrophils seems to be associated with the expression of *cagA* (cytotoxicity associated gene A) and other genes within the pathogenicity island [21]. *H. pylori* infections are also associated with local increase in IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and there is a correlation between the overproduction of these cytokines and a high-grade mucosal inflammation [22].

*Helicobacter pylori* is a genetically diverse species [23]. The strains isolated from different hosts present various virulence capacity. The bacteria containing *cagA* more frequently induce severe gastritis, gastric ulcer, atrophic gastritis and gastric cancer [24]. *Helicobacter pylori* isolated from one individual may express LeX or LeY or both determinants, suggesting a population of clone variants colonizing gastric mucosa [12]. It has been suggested that the release of LPS from *H. pylori* in the site of the infectious niche may play an important role in inducing a local or systemic inflammatory response. In this study we asked whether LeX and LeY determinants of *H. pylori* LPS may influence the production of inflammatory cytokines such as TNF- $\alpha$  and IL-8. We showed that the lack of Lewis determinants in *H. pylori* LPS resulted in a significant reduction of its ability to induce TNF- $\alpha$  secretion by peripheral blood mononuclear leukocyte cultures as compared to the activity of *H. pylori* LPS containing LeXY determinants as well as to the activity of standard *E. coli* LPS. A similar tendency has been shown for IL-8 secretion but only concerning PBML from *H. pylori* negative donors. This may confirm that both bacterial and host factors might be important for the development and maintenance of the inflammatory response during *H. pylori* infections. Tumour necrosis factor  $\alpha$  and IL-8 were produced mainly by monocyte-enriched fraction of PBMC, in response to *H. pylori* LPS of LeXY type. Two LPS binding sites were needed for TNF- $\alpha$  and IL-8 production, which was shown by inhibition of these cytokines' secretion in PBML cultures pre-treated with monoclonal anti-LBP and anti-CD14 antibodies before stimulation of PBML with *H. pylori* LPS of LeXY type, as compared to antibody untreated cells. The role of LeXY determinants of *H. pylori* LPS during the course of *H. pylori* infections remains unclear. It has been suggested that the host Lewis phenotype may select *H. pylori* expressing Lewis antigens based on the similarities between LeX and Le<sup>a</sup> or LeY and Le<sup>b</sup> determinants [25].

It has also been shown that *H. pylori* LPS diminished ingestion of *H. pylori* by human polymorphonuclear leukocytes (PMNs) [26]. Lipopolisaccharides driven inhibi-

tion of *H. pylori* ingestion by PMNs was neutralized by recombinant LPS-binding protein (LBP), but it was Lewis antigen independent although LBP was found to interact in the ELISA assay with *H. pylori* LPSs with LeX or LeXY but not with LPS lacking Lewis determinants. Thus, LPS/LBP binding could be significant for activation of PMNs via CD14 receptors resulting in the release of inflammatory cytokines rather than phagocytosis of the bacteria [26]. It is not possible to exclude a possibility that such phenomena could prevent the eradication of *H. pylori* bacteria and increase the inflammation that is providing bacteria with nutritive compounds. The *H. pylori* LPS has a much lower endotoxic activity, and also a lower ability to stimulate macrophages to produce proinflammatory cytokines, nitric oxide prostaglandins as compared to *E. coli* LPS. The low biological activity of *H. pylori* LPS is related to the modification of lipid A, which is the only region of LPS to be recognized by the innate system via the LPS receptor – the TLR4-MD2-CD14 complex, which is present on macrophages, granulocytes and dendritic cells [27]. It has also been shown that *H. pylori* LPS induces cell activation rather through TLR2 but not TLR4 receptor and that LPS from some *H. pylori* strains may antagonize TLR4 [28]. However, in the light of the presented results, the influence of LeX and LeY determinants expressed in *H. pylori* LPS on the production of inflammatory cytokines by the host cells cannot be excluded. Recently, Grebowska *et al.* [29] have shown that both in patients with coronary heart syndrome (CAD) and in healthy subjects infected with *H. pylori*, the level of antibodies to LPS of LeXY(+) type was higher than to LPS of LeX(+) or LeXY(-) type, thus indicating that LPS of LeXY(+) type was most effective in the stimulation of humoral response, potentially self-destructive, in the CAD *H. pylori* positive patients. Variations in *H. pylori* LPS may facilitate the bacteria to evade the innate immune component surfactant protein D. This phenomenon is linked to changes in the fucosylation of the O chain, which was concomitant with slipped-strand mispairing in a poly (C) tract of the fucosyltransferase A (*fuc T1*) gene [13]. It has been suggested that *H. pylori* employs similar mechanisms compared to other pathogens or commensals to achieve evasion or suppression of the innate immune system including an increase or inhibition of responses [30]. *Helicobacter pylori*, similarly to many intestinal colonizers, developed mechanisms to vary its LPS chemistry in order to subvert the recognition by innate immune receptors [11].

In conclusion, we have demonstrated that *H. pylori* LPS, especially of the Lewis XY(+) type *in vivo*, might be involved in LPS signalling for inflammatory cytokines and in this way regulate the outcome of *H. pylori* infections and bacterial load.

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