Polish Journal of Microbiology
2008, Vol. 57, No 3, 185–192

ORIGINAL PAPER

Anti-phagocytic Activity of *Helicobacter pylori* Lipopolysaccharide (LPS) – Possible Modulation of the Innate Immune Response to these Bacteria

ANETA GRĘBOWSKA[1], ANTHONY P. MORAN[2], AGNIESZKA MATUSIAK[1], LEOKADIA BĄK-ROMANISZYN[3], ELŻBIETA CZKWIANIANC[3], TOMASZ RECHCIŃSKI[4], MARIA WALENCKA[1], IZABELA PŁANETA-MAŁECKA[3], WIESŁAWA RUDNICKA[1], and MAGDALENA CHMIELA[1*]

[1] Department of Immunology and Infectious Biology, University of Łódź, Łódź, Poland
[2] National University of Galway, Galway, Ireland, [3] Mother Health Center in Łódź, Łódź, Poland
[4] II Cardiology Clinic, Medical University in Łódź, Łódź, Poland

Received 14 April 2008, revised 7 July 2008, accepted 21 July 2008

Abstract

The *Helicobacter pylori* infections are followed by an infiltration of the gastric mucosa by neutrophils and macrophages. Accumulation of phagocytes enables them to interact with *H. pylori*, but a great number of infected subjects cannot eradicate these bacteria. The *H. pylori* inhibits its own uptake by blocking the function of phagocytes. The anti-phagocytic mechanism depends on bacterial surface structures and the presence of the *cag* pathogenicity island (PAI). The role of *H. pylori* lipopolysaccharide (LPS), during phagocytosis of these bacteria is not clear. LPS may mediate direct bacteria/phagocyte interactions and it may also regulate antibacterial activity of the phagocytes. In this study we investigated the influence of *H. pylori* LPS on phagocytosis of these bacteria. The *H. pylori* LPS inhibited an ingestion of these microbes by human peripheral blood granulocytes. This was correlated with a diminished ability of phagocytes to reduce MTT-tetrazolium salt. The anti-phagocytic effect of *H. pylori* LPS was reduced by recombinant lipopolysaccharide binding protein (rLBP). It is possible that *in vivo H. pylori* LPS may diminish elimination of these bacteria from the gastric mucosa promoting an infection persistence. However, LBP may modulate the uptake of *H. pylori* due to neutralization of anti-phagocytic effect of its LPS.

Key words: *H. pylori*, LPS, LBP, phagocytosis

Introduction

Colonization of the gastric mucosa with *Helicobacter pylori* results in the development of chronic gastritis and for a subset of patients, progression of chronic gastritis to ulcer or cancer is observed (Blaser and Berg, 2001; Hatakayema and Brzozowski, 2006). Host recognition of microbial invasion promotes an inflammatory response through the secretion of antimicrobial compounds and recruitment of phagocytic cells such as macrophages and neutrophils. This initial response to the pathogens called innate immunity requires the recognition of evolutionary conserved microbial patterns including flagella, lipoproteins, peptidoglycan and LPS (Kawai and Akira, 2005). The mechanisms of pathologic changes induced by *H. pylori* involve an activation of NFκB which is followed by the release of the proinflammatory cytokines and an overexpression of growth factors (Neuman *et al*., 2006; Suganuma *et al*., 2006). However, an acute inflammatory response may be detrimental both to the host and to *H. pylori.* It has been hypothesized that the strains that do not strongly activate the host response have been selected (Blaser, 2005). *H. pylori* may modulate expression of genes encoding defensins, in a *cag* dependent manner (cag-cytotoxin associated gene) (Boughan *et al*., 2006). An important anti-inflammatory mechanism of *H. pylori* is the scavenging activity of the reactive oxygen species (ROS) due to catalase, superoxide dismutase and alkyl hydroperoxidase activity which is increased in *cag*-positive *H. pylori* strains (Briede *et al*., 2005). The presence of *mutS* homoloque DNA mismatch repair system may also play an important role (Wang *et al*., 2005). Other mechanisms for evading immune recognition involve antigenic variation of surface components,

* Corresponding author: M. Chmiela, Department of Immunology and Infectious Biology University of Łódź, Banacha 12/16, 90-237 Łódź, Poland; phone: (48) 42 6354186; **e-mail: chmiela@biol.uni.lodz.pl**

including outer membrane proteins and LPS antigens (Appelmelk *et al*., 1999). LPS from most bacterial organisms serves as a potent signal for the development of inflammation. An important *H. pylori* adaptation is the synthesis of LPS that is less proinflammatory than LPSs from typical enterobacterial species (Khamri *et al.*, 2005). The *H. pylori* LPS has a much lower endotoxic activity, and also a lower ability to stimulate macrophages to produce of proinflammatory cytokines, nitric oxide prostaglandins as compared to *E. coli* LPS (Muotiala *et al*., 1992). The low biological activity of *H. pylori* LPS is related to the modification of Lipid A (Moran and Aspinall, 1998), which is the only region of LPS to be recognized by the innate immune system *via* LPS receptor – the TLR4-MD2-CD14 complex, which is present on macrophages, granulocytes and dendritic cells. Analyses of the interactions of purified *H. pylori* LPS with TLRs suggest that, in contrast to LPSs from most other gramnegative bacteria, *H. pylori* LPS is not well recognized by TLR4 (Smith *et al*., 2003; Ferrero, 2005) and may decrease the cell surface expression of this receptor and a simultaneous decrease in cytokine production (Nomura *et al*., 2000; Medvedev *et al*., 2000). Another feature of *H. pylori* LPS is the expression of O antigens that mimic Lewis blood group antigens present on human cells. Such a mimicry may allow the bacteria to avoid the immune recognition due to the similarity to the host antigen (Aspinall and Monteiro, 1996).

Despite the recruitment of neutrophils and macrophages to the gastric mucosa, during *H. pylori* infections, these bacteria are not efficiently controlled by phagocytes. It has been shown that *H. pylori* can alter phagocyte function. Several mechanisms allow these bacteria to evade opsonization, actively retard phagocytosis and affect membrane trafficking and phagosome maturation. Some strains may also evade intracellular killing (Allen *et al*., 2000, Allen 2007). Such *H. pylori* antiphagocytic behaviour depends on the presence of the *cag* pathogenicity island (PAI) in its genome, and only the type I strains which contain the PAI are able to inhibit their uptake by monocytes and granulocytes (Romarao *et al*., 2000).

Engulfment of bacterial cells by phagocytes is a receptor-directed process. *H. pylori* attachment to phagocytes is mediated by a combination of sialic acid-specific and sialic acid-independent adhesins and heparan sulphate binding proteins (Chmiela *et al*., 1997). Recently the adhesins that bind to lactosyloceramides (HpaA) or to sialyl-Lewis X (SabA), or to as yet unknown sialic acid containing glycoproteins (HP071), have been established (Mahdavi *et al*., 2002; Bennett and Roberst, 2005; Fantini *et al*., 2006). Engulfment of *H. pylori* may also be modulated by extracellular matrix proteins such as laminin, vitronectin, type IV collagen, heparan sulphate and hyaluronic acid (Ljungh *et al*., 1996). Attachment to laminin is mediated by both SabA and LPS (Valkonen *et al*., 1994; Waltz *et al*., 2005). *H. pylori* LPS may cause its various effects when released to the mucosal environment due to microbial autolysis. In this study we addressed a question how *H. pylori* LPS may influence the engulfment of these bacteria by granulocytes infiltrating the gastric mucosa in response to *H. pylori* infection and limit inflammatory reaction of the host. The potential role of lipopolysaccharide binding protein (LBP), which binds and facilitates LPS transportation, has also been included as a possible modulator of *H. pylori* phagocytosis. The influence of LBP alone or in combination with *H. pylori* LPS to binding/ingestion of these bacteria by peripheral blood polymorphonuclear leukocytes – PMNs has been estimated. Moreover, the level of LBP in the sera of *H. pylori* infected and uninfected subjects has been compared.

Experimental

Materials and Methods

Subjects. The experimental study group consisted of: 1) 27 patients with chronic dyspeptic symptoms due to *H. pylori* infection – H. p. (+), confirmed by endoscopy-based methods: detection of urease activity and the presence of *Helicobacter*-like organisms in the biopsy specimens; 2) 60 healthy control group (HC) seronegative (34) or seropositive (26) to anti-*H. pylori* antibodies, estimated by ELISA with a glycine acid extract (EG) from the reference *H. pylori* strain, as previously described (Rechciński *et al*., 1997). For phagocytosis study PMNs from three healthy subjects, seronegative for anti-*H. pylori* antibodies, to avoid immune opsonization, have been used. The study was approved by the local Ethical Committee. All participants signed informed consent.

Bacterial strain and culture conditions. The *H. pylori* G33 type I strain (*cagA*+/*vacA*+) was obtained from Instituto Ricerche Immunobiologische – IRIS, Siena, Italy. The bacteria were stored at –70°C in tryptic soy broth containing 10% glycerol. Before being used in experiments the bacteria were cultured for 48 h, at 37°C in microaerophilic conditions on blood agar containing 10% heat inactivated fetal calf serum. *H. pylori* G33 strain was of Lewis XY type. The expression of Lewis (Le) determinants on *H. pylori* cells was estimated by ELISA using monoclonal anti-LeX and anti-LeY antibodies (Seikagaku, Tokyo, Japan) and synthetic LeX and LeY antigens as controls (Dextra Lab, Reading, UK), as previously described (Rudnicka *et al*., 2001).

FITC labelling of *H. pylori* bacteria. The *H. pylori* bacteria collected from the plates were washed

once with PBS and resuspended in a buffer containing 100 μg/ml fluoresceine isothiocyanate (FITC, Sigma, St Louis, Michigan, US). The mixture was agitated for 30 min at room temperature. The bacteria, after extensive washing with PBS, were resuspended in PBS containing 4% BSA, to bind unconjugated FITC to BSA. After incubation, 15 min at room temperature, the bacteria were washed and resuspended at 1×10^6 cells/ml in RPMI-1640 medium. The effectiveness of the labelling was controlled by preparing the standard curve for qantitation of FITC labelled bacteria. Serially diluted bacterial cell suspensions in RPMI-1640 medium (from 1×10^5 to 1×10^9 cells/ml) were distributed into the wells and the fluorescence of bacteria was measured. The values of fluorescence were plotted as a function of the number of bacteria (Chmiela *et al*., 1997).

Interaction of LBP with *H. pylori*. The interaction of *H. pylori* cells and their LPS with human recombinant LBP (rLBP, HyCult Biotechnology, Uden, The Netherlands) was estimated by ELISA on microplates coated for 18 h, at 4°C with 100 μl/well of *H. pylori* bacterial suspensions (1×10^7 cells/ml), *H. pylori* LPS (1 μg/ml) or rLBP 1 μg/ml (control well), always in phosphate buffered saline – PBS, pH 7.4. The concentrations of LPS and rLBP were selected by titration. Unbound plastic was blocked with PBS, with 0.1% bovine serum albumin (PBS/BSA). After washing the plates 4 times with PBS/0.05% Tween 20 (PBS/Tween), the rLBP (5 μg/ml in PBS/BSA, 100 μl/well, HyCult, Uden, The Netherlands) was added to the wells coated with bacteria or LPS, the buffer was added to the wells coated with rLBP, and the plates were incubated for 2 h, at 37°C. After washing, the plates were incubated for 2 h, at 37°C with mouse monoclonal antibodies against recombinant human LBP (10 μg/ml, HyCult), and then for 1 h with rabbit antibodies against mouse immunoglobulins labeled with horseradish peroxidase-HRP (Dako, Glostrup, Denmark). The color reaction was developed in the presence of substrate solution: 1mg o-phenylenediamine dihydrochloride – OPD (Sigma, St Louis, Michigan, US) in 1 ml citric-acid phosphate buffer, pH 5.0, supplemented with 0.5 μl/ml of 30% H_2O_2. The reaction was stopped with citric acid and the absorbance was measured at 450 nm using Victor 2 reader (Wallac, Oy, Turku, Finland). In every ELISA the control wells were used for excluding unspecific reactions.

Phagocytosis. Polymorphonuclear leukocytes (PMNs) from three healthy subjects, seronegative to anti-*H. pylori* antibodies, were separated from fresh blood collected by veinpuncture with heparin as an anticoagulant, by Polymorphoprep gradient centrifugation (Nycomed, Oslo, Norway). For phagocytosis, PMNs suspensions (1×10^6 cells/ml) were prepared in 1 ml volume of RPMI-1640 with gentamycin (5 μg/ml) containing: 1) rLBP (10 ng/ml); 2) *H. pylori* LPS (10 or 100 ng/ml); 3) LBP (10 ng/ml) and *H. pylori* LPS (10 or 100 ng/ml) or 4) no stimulant. The cells suspended in an appropriate medium were added in triplicate to the wells of microplate (100 μl/well) and supplemented with FITC-labeled bacteria in RPMI-1640, at the ratio 1:10 or 1:100, and then incubated for 30 min, 1 h or 2 h, at 37°C, 5% CO_2. The phagocytes to the bacteria ratio 1:100 and 1h incubation time were found to be optimal for the estimation of *H. pylori* uptake by PMNs (Chmiela *et al*., 1997). Phagocytosis was stopped on ice. The unbound bacteria were removed by washing the cells with ice-cold PBS with gentamycin (PBS/G). The extracellular fluorescence was quenched with crystalline violet (500 g/ml in PBS). The dye was exchanged with PBS/G. The intensity of fluorescence was measured using Victor 2 counter (Wallac, Oy, Turku, Finland) and expressed in relative fluorescence units (RFU) – fluorescence counts. The wells containing FITC labeled bacteria alone were used as a control of quenching effectiveness.

The PMN's viability assays. The viability of the cells was estimated on the basis of their ability to reduce MTT tetrazolim salt or the activity of their mitochondrial dehydrogenase, which may indicate the appearance of early apoptotic changes in the cells. The cells (2×10^6/ml) were incubated in the presence or absence of *H. pylori* LPS (10–100 ng/ml), for 2 h, 37°C, 5% CO_2. The plates were centrifuged for 5 min, 250×g and washed twice in RPMI-1640. The Cell Proliferation and Viability Assay, TACS™ MTT Assay (R&D System, Minneapolis,US), and Mito Light™ Apoptosis Detection Kit (Chemicon International, Temecula, Canada) were used as recommended by the manufacturers. The intensity of MTT reduction was estimated spectrophotometrically at 600 nm wave length. The cell viability was expressed as optical density units OD_{600}. There was a correlation between the viable cell number, used for preparing the standard curve, and the absorbance intensity. The enhancement of green versus yellow/red fluorescence, distinguishing apoptotic from live cells, respectively, was measured using Victor 2 reader and 480/530 excitation/emission filters.

The serum LBP concentration. Blood samples from antecubical vein were obtained from all study participants for serological tests. The sera were stored at –70°C. The commercial Enzyme Linked Immunosorbent Assay – ELISA kit for estimation of LBP (HyCult Biotechnology) was used as recommended by the manufacturer (HyCult Biotechnology).

Statistics. Statistica 5.5 PL program with non-parametric tests was used: Mann-Whitney U test (for impaired data) to verify the hypothesis that two analyzed samples came from two statistically different populations; Chi-square χ^2 test for the comparison of the prevalence of analyzed parameters in the studied groups.

Results

Phagocytosis of *H. pylori* bacteria by human blood PMNs in the presence or absence of *H. pylori* LPS and/or rLBP. The engulfment of FITC-labeled *H. pylori* bacteria by PMNs from healthy donors, seronegative for anti-*H. pylori* IgG, in the medium containing 10 or 100 ng/ml *H. pylori* LPS, was diminished as compared to the ingestion of bacterial cells in the RPMI-1640 medium alone (Fig. 1). The inhibitory effect was dose dependent. The ingestion of *H. pylori* bacteria observed for the PMN cultures in medium alone, RFU 5463 ± 1084, was decreased to the value of RFU 4352 ± 535 and RFU 3146 ± 357 in the cultures with, respectively, the lower and higher concentration of *H. pylori* LPS. This anti-phagocytic effect of *H. pylori* LPS was reversed by human rLBP used in a low concentration of 10 ng/ml. The rLBP alone did not influence phagocytosis of *H. pylori* bacteria. The engulfment of *H. pylori* bacteria in the cultures with (RFU 5199 ± 636) and without rLBP (RFU 5463 ± 1084) was on the same level. The *H. pylori* LPS molecules were responsible for the binding of rLBP to the bacterial surface. It has been showed by a similar reactivity of monoclonal anti-LBP antibody with whole *H. pylori* bacteria and *H. pylori* LPS in solution, by ELISA, $p > 0.05$ (Fig. 2).

The cell viability study. The effect of *H. pylori* LPS on PMNs was determined by three standard cytotoxicity methods: the MTT assay, MitoLight assay and

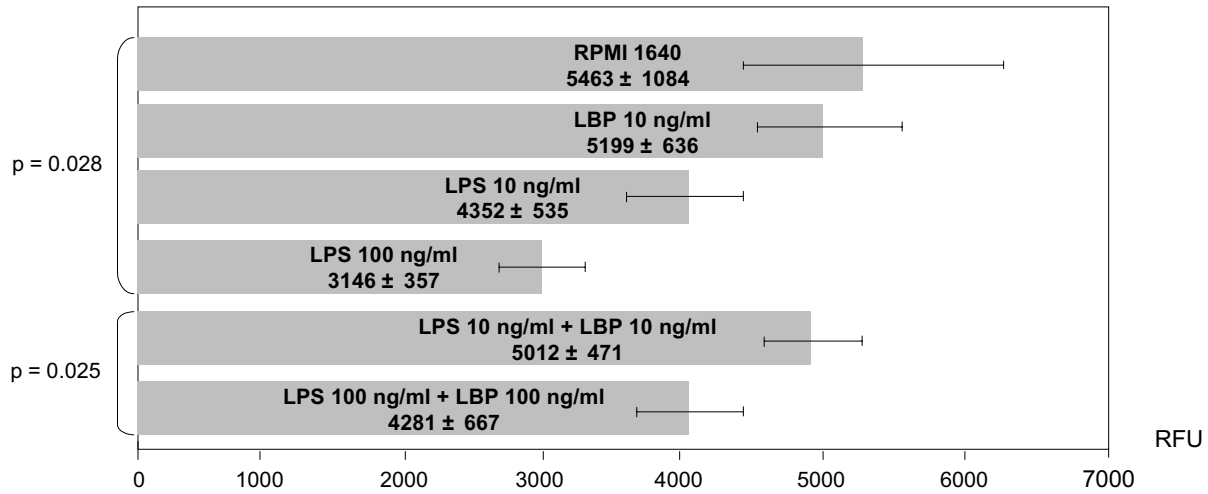

Fig. 1. The intensity of phagocytosis of FITC-labelled *H. pylori* bacteria by human granulocytes in the presence or absence of *H. pylori* LPS and/or rLBP. RFU-relative fluorescence units.

The results from five experiments were analyzed. The difference was statistically significant when $p < 0.05$ (U Mann-Whitney test).

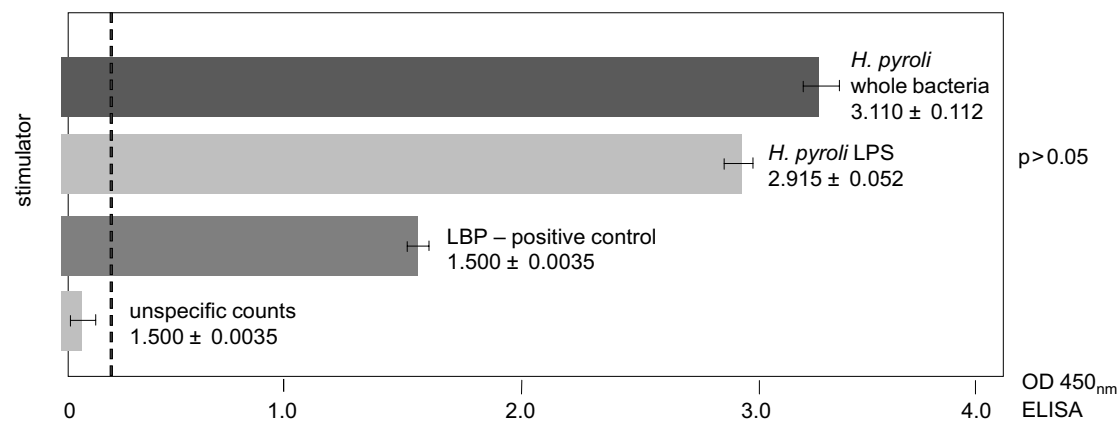

Fig. 2. Interaction of recombinant lipopolysaccharide – binding protein (rLPB) with *H. pylori* whole bacteria and *H. pylori* LPS, estimated by ELISA.

The plates were coated using *H. pylori* whole bacterial cells inactivated in PBS+0.01% sodium azide, *H. pylori* LPS or rLBP. Mouse monoclonal anti-rLBP antibodies and then rabbit antibodies against mouse immunoglobulins bound with HRP were used for detection of LBP binding in the ELISA for detection of LBP binding. The ELISA cut off was a double OD_{450} count ± SD from the wells coated with *H. pylori* whole bacteria or *H. pylori* LPS incubated with HRP antibodies. The results were expressed as mean arithmetic values ($\bar{x}$) ± SD. The mean values were considered significant when $p < 0.05$.

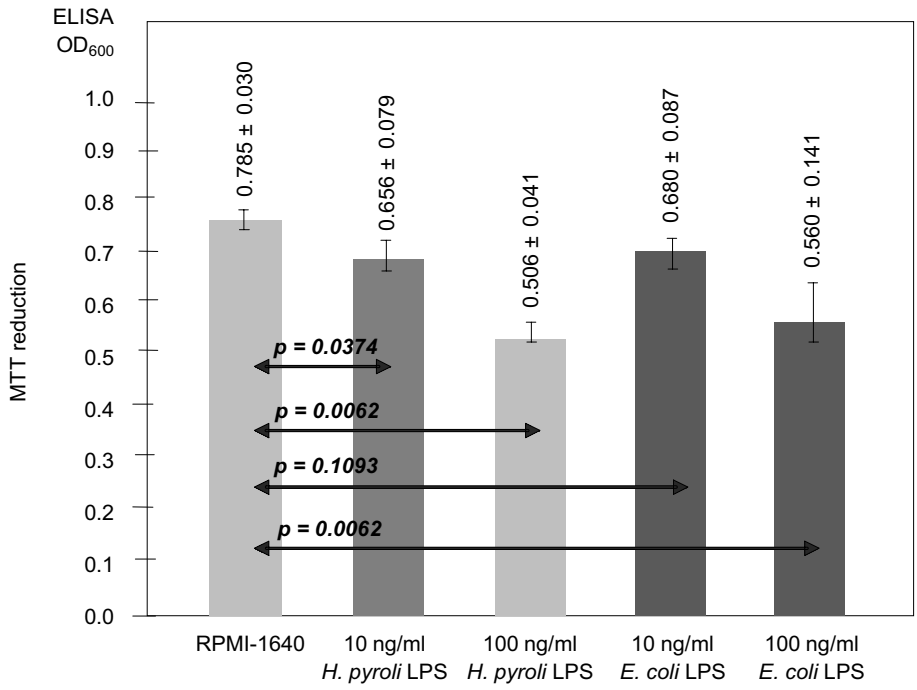

Fig. 3. The intensity of MTT reduction (OD_{600}) by human polymorphonuclear cells (PMNs) in the presence or absence of *H. pylori* or *E. coli* LPS.

The PMNs were incubated for 2 h at 37°C, 5% CO_2 in the RPMI-1640 medium alone or such medium supplemented with 10 or 100 ng/ml of *H. pylori* of *E. coli* LPS. The MTT reduction was estimated spectrophotometrically (OD_{600}) using the cell proliferation and viability kit, TACS™ MTT (R & D System), as recommended by the manufacturer. The MTT reduction was expressed as mean arithmetical values ($\bar{x}$) ± SD, which were compared using the U Mann-Whitney test. The values were considered significant when $p<0.05$.

trypan blue dye exclusion test. Data on Fig. 3 show that *H. pylori* LPS significantly diminished the reduction of MTT by PMNs. The MTT reduction by PMNs in the cultures without LPS, OD_{600} 0.758 ± 0.031, decreased to OD_{600} 0.656 ± 0,079 ($p = 0.0374$) and OD_{600} 0.506 ± 0.041 ($p = 0.0062$) in the cultures, respectively with 10 ng/ml and 100 ng/ml *H. pylori* LPS. It is important that *H. pylori* LPS driven inhibition of the MTT reduction by PMNs was in the same range as the decrease in the MTT reduction caused by *E. coli* LPS (Fig. 3). This fact was interesting as regards a current opinion on much stronger endotoxicity of *E. coli* LPS in comparison with *H. pylori* LPS. The *H. pylori* induced inhibition of the reduction of MTT tetrazolium salt observed in MTT assay was not confirmed in the performed simultaneously MitoLight test. There was no difference between the mitochondrial membrane potential measured by use of MitoLight assay in PMNs incubated for 2 h, 37°C, 5% CO_2, in the medium with or without *H. pylori* LPS (10 ng/ml and 100 ng/ml) (data not shown). At the same time, the cell viability estimated by trypan blue exclusion was in the range of 96–98% in PMN cultures, with and without *H. pylori* LPS, excluding its lysis activity in our experimental model.

The serum LBP concentration. The levels of LBP in *H. pylori* positive patients with gastritis (10.400 ± ± 4.413 mg/ml) and in healthy *H. pylori* uninfected volunteers were similar (10.640 ± 8.533 μg/ml) (Table I). There was no statistically significant difference ($p>0.05$) in LBP concentration between healthy subjects, seronegative or seropositive for anti-*H. pylori* glycine extract IgG, although the medium LBP level was higher in seropositive donors (17.740 ± ± 17.130 μg/ml versus 10.640 ± 8.533 μg/ml). In a previous study Grebowska *et al*. (2006) showed, that the antibodies reacting with *H. pylori* LPS immobilized to ELISA plastic plates were present in the sera from all volunteers, with and without gastric disorders, with and without *H. pylori* infection, seropositive and

Table I
The serum LBP concentration

Group	(n)	LBP (μg/ml) ($\bar{x}$ ± SD)
H. pylori (+) – seropositive gastritis	(27)	10.400 ± 4.413
healthy	(30)	17.740 ± 17.130
H. pylori (+) – seropositive healthy	(30)	10.640 ± 8.533
Significance		$p>0.05$

The concentration of LBP in the serum samples was estimated by commercial ELISA test (HyCult, Biotechnology, Uden, The Netherlands) as recommended by the manufacturer. The results were expressed as mean arithmetic values ($\bar{x}$) and standard deviation (SD). The mean values were compared using the U Mann-Whitney test. The values were considered significant when $p<0.05$.

seronegative for anti-*H. pylori* glycine extract IgG. This common reactivity of human sera with *H. pylori* LPS in ELISA resulted from the cross reactivity of LPSs synthesized by Gram-negative bacteria not related to *H. pylori*. However, the medium level of anti-*H. pylori* antibodies was higher in the sera from *H. pylori* infected gastric patients than in the sera of control groups.

Discussion

An abundant granulocyte infiltration of gastric mucosa is a common feature of *H. pylori* infections. However, these professional phagocytes are not capable of clearing the infection which may persist for life (Lee and Josenhans, 2005). It has been shown that *H. pylori* bacteria evade intracellular killing in the process dependent on the *cag* pathogenicity island (Allen *et al*., 2000; Romarao *et al*., 2000). The ingestion of bacteria is also modulated by extracellular matrix proteins (Ljungh *et al*., 1996; Chmiela *et al*., 1997; Mahdavi *et al*., 2002; Bennet and Roberts, 2005; Fantini *et al*., 2006). In this study we were interested in how *H. pylori* LPS may influence the course of phagocytosis process of these bacteria. First, we demonstrated that *H. pylori* engulfment by granulocytes was significantly less efficient in the presence of *H. pylori* LPS than in the medium alone. The antiphagocytic activity of *H. pylori* LPS was dose dependent. Our finding may suggest that during autolysis of *H. pylori* cells, *in vivo*, the released LPS may help the live bacteria to avoid phagocytosis. Thus, the infection persists for a long time. It should be mentioned that *H. pylori* bacteria use several mechanisms to disrupt the functioning of granulocytes. In the previous study we showed that these bacteria may avoid phagocytosis by strong binding of the host compounds containing sialic acid, heparin, heparan sulphate and vitronectin in the presence of complement (Chmiela *et al*., 1997). Strapagiel *et al*. (2006) showed that natural mannose-binding lectin (MBL) down regulated phagocytosis of *H. pylori*. The ingestion of *H. pylori* bacteria in the medium with human serum depleted in MBL was more intensive than in the medium with complete serum containing MBL.

A central role in the innate immune response to Gram-negative bacterial pathogens is played by lipopolysaccharide – binding protein (LBP) which is primarily synthesized by hepatocytes but also by epithelial and muscle cells (Zweigner *et al*., 2006). It has been proposed that LPS molecules are recognized by a complex cascade of extracellular pattern recognition receptors, which shuttles the LPS aggregates or LPS from the bacterial membrane to CD14, where the acyl chain of lipid A is protected from the solvent in the hydrophobic binding pocket of CD14 (Jerala, 2007). The CD14 transfers the LPS to MD-2 protein and the LPS-MD2 complex triggers signaling cascade *via* Toll-like receptor 4 (TLR4) (Prohinar *et al*., 2007). Lepper *et al*., (2005) has shown that *H. pylori* LPS induced cell activation through TLR2 and LPS from some *H. pylori* strains is able to antagonize TLR4. It has also been suggested that TLR signaling plays an important role during phagocytosis (Underhill and Gantner, 2004). In this paper, rLBP at a low concentration of 10 ng/ml, neutralized anti-phagocytic effect of *H. pylori* LPS (Fig. 1). By use of anti-rLBP monoclonal antibody, we demonstrated that whole *H. pylori* bacteria and isolated *H. pylori* LPS bound almost the same amount of rLBP. Thus, we can carefully speculate that in *H. pylori* colonized stomach the LBP may prevent anti-phagocytic effect of outer bacterial membrane LPS and LPS in solution. It has been estimated that LBP has a dual role in the interactions with LPS. At the low concentration it enhances the LPS signaling by removing it from the bacterial membranes and transferring the monomers of LPS to the CD14 (Yu and Wright, 1996; Vesy *et al*., 2000). At the high concentration LBP inhibits the LPS signaling by shuttling the LPS to the serum lipoprotein and by a formation of aggregates with LPS (Wurfel *et al*., 1995; Gutsman *et al*., 2001). Thus, an increased secretion of LBP stimulated by *Enterobacteriaceae* LPS has been found in the serum of septic patients. In this study, a similar concentration of LBP was detected in the sera from *H. pylori* infected patients with gastritis and healthy subjects both *H. pylori* infected and uninfected. A restricted to the stomach extent of *H. pylori* infection as well as unique structural features of *H. pylori* LPS (Cunningham *et al*., 1996; Moran and Aspinall, 1998) may determine a lack of its systemic endotoxic effects and redundancy of the increased serum LBP level, in *H. pylori* symptomatic or asymptomatic infections.

The *H. pylori* LPS driven decrease in the engulfment of *H. pylori* bacteria by PMNs was associated with a lower viability of the phagocytes showed in MTT assay. The intensity of MTT reduction by PMNs incubated for 2 h with 10 or 100 ng/ml *H. pylori* LPS was significantly lower ($p = 0.0374$ and 0.0062 respectively) than the MTT reduction by the cells in RPMI-1640 medium. In MTT test, the incubation of the cells with MTT leads to formazan crystals developing in living and early apoptotic cells. Dead cells do not produce formazan crystals. Thus, our results could have suggested that *H. pylori* LPS driven apoptotic changes in PMNs were responsible for their weakening phagocytic activity. However, in this study the diminished reduction of MTT by granulocytes in the medium containing *H. pylori* LPS was not correlated with the enhancement of apoptotic features in the cells, estimated on the basis of mitochondrial mem-

brane potential in MitoLight test or by trypan blue exclusion assay. To explain the discrepancy in the results of three currently used cytotoxic assays, chosen by us for the estimation of the effects of *H. pylori* LPS in PMNs cultures, different parameters like tested compounds, detection mechanism, specificity and sensitivity of the assays have to be considered. Thus, the MitoLight assay could be not appropriate for the measurement of the early apoptotic changes in PMNs. On the other hand, it is worth mentioning that granulocytes are short-living cells that have a capability of spontaneous apoptosis. Thus, the time maintenance of the PMNs in the cultures has to be rather short; in this paper it was 2 h. As it was published by Hofman *et al.* (2001) and Turina *et al.* (2005), *H. pylori* LPS delays the programmed cell death of granulocytes, and this may influence the severity of inflammation in the gastric mucosa.

In conclusion we have demonstrated that *H. pylori* LPS diminishes the engulfment of *H. pylori* bacteria by PMNs. The effect is dose dependent and associated with the decrease in the viability of phagocytes. Thus, LPS should play a critical role in the persistence of *H. pylori* infections despite the abundant granulocyte infiltration of gastric mucosa. The LBP, possibly produced locally by epithelial cells, neutralizes anti-phagocytic activity of LPS and might limit the growth of the bacteria and a bacterial load dependent inflammation.

Acknowledgements

Supported by Polish Ministry of Science and Higher Education (grant N401 02131/0379)

Literature

Allen L.A.H., L.S. Schlesinger and B. Kang. 2000. Virulent strain of *Helicobacter pylori* demonstrate delayed phagocytosis and stimulate homotypic phagosome fusion in macrophages. *J. Exp. Med*. **191**: 115–127.

Allen L.A. 2007. Phagocytosis and persistence of *Helicobacter pylori*. *Cell. Microbiol*. 9: 817–828.

Appelmelk B.J., S.L. Martin, M.A. Monteiro, C.A. Clayton, C.H. Hokke, M.B. Perry, C.M. Vandenbrouche-Grauls and J.G. Kusters. 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α-fucosylotransferase genes. *Infect. Immun*. 67: 5361–5366.

Aspinall G.O. and M.A. Monteiro. 1996. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. *Biochemistry* 35: 2498–2504.

Bennet M.J. and I.S. Roberts. 2005. Identification of a new sialic acid-binding protein in *Helicobacter pylori. FEMS Immunol. Med. Microbiol*. 44: 163–169.

Blaser M.J. 2005. The biology of *cag* in *Helicobacter pylori* – human interaction. *Gastroenterology* 128: 1512–1515.

Blaser M.J. and D.E. Berg. 2001. *Helicobacter pylori* genetic diversity and risk of human disease. *J. Clin. Invest.* 107: 767–773

Boughan P.K., R.M. Argent, M. Body-Malapel, J.H. Park, E.K. Erwings, A.G Bowie, S.J. Ong, S.J. Cook, O.E. Sorensen, B.A. Manzo and others. 2006. Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of beta-defensis during *Helicobacter pylori* infection. *J. Biol. Chem*. 281: 11637–11648.

Briede J.J., R.G. Pot, E.J. Kuipers, A.H. van Vliet, J.C. Klejnjens and J.G. Kusters. 2005. The presence of *cag* pathogenicity island is associated with increased superoxide anion radical scavenging activity by *Helicobacter pylori*. *FEMS Immunol. Med. Microbiol*. 44: 227–232.

Chmiela M., E. Czkwianianc, T. Wadstrom and W. Rudnicka. 1997. Role of *Helicobacter pylori* surface structures in bacterial interaction with macrophages. *Gut* 40: 20–24.

Cunningham M.D., C. Seachord, K. Ratchiffe, B. Bainbrridge, A. Amffo and R.P. Darveau. 1996. *Helicobacter pylori* and *Porphyromonas gingivalis* lipopolysaccharides are poorly transferred to recombinant soluble CD14. *Infect. Immun*. 64: 3601–3608.

Fantini J., N. Garmy and N. Yahi. 2006. Prediction of glycolipid-binding domains from the amino acid sequence of lipid raft-associated proteins: application to HpA, a protein involved in he adhesion of *Helicobacter pylori* to gastrointestinal cells. *Biochemistry* 45: 10957–10962.

Ferrero R.L. 2005. Innate immune recognition of the extracellular mucosal pathogen *Helicobacter pylori*. *Mol. Immunol.* 42: 879–885.

Grebowska A., T. Rechcinski, A. Moran, E. Czkwianianc, L. Bak-Romaniszyn, A. Owczarek, M. Dziuba, M. Kowalewicz-Kulbat, M. Druszczynska, M. Krzeminska-Pakula and others. 2006. Increased levels of immunoglobuline and non-immunoglobuline markers of host response to *H. pylori* LPS in the patients with coronary artery disease (CAD). *Curr. Trends Immunol*. 7: 85–96.

Gutsmann T., M. Muller., S. F. Caroll., R. C. MacKenzie and U. Weydel. 2001. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. *Infect. Immun*.69: 6942–6950.

Hatakayema M. and T. Brzozowski. 2006. Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* 11 (Suppl.1): 14–20.

Hofman V., V.Ricci, B. Mograbi, P. Brest, F. Luciano, P. Boquet, B. Rossi, P. Auberger and P. Hofman. 2001. *Helicobacter pylori* lipopolysaccharide hinders polmorphonuclear leucocyte apoptosis. *Lab. Invest.* 81: 375–384.

Jerala R. 2007. Structural biology of the LPS recognition. *J. Med. Microbiol*. 297: 353–363.

Kawai, T. and S. Akira. 2005. Pathogen recognition with Toll-like receptor. *Curr. Opin. Immunol*. 17: 338–344.

Khamri W., A.P. Moran, M.L. Worku, Q.N. Karim, M.M. Walker, H. Annuk, J.A. Ferris, B.J. Appelmelk, P. Eggleton, K.B. Reid and others. 2005. Variations in *Helicobacter pylori* lipopolysaccharide to evade the innate immune component surfactant protein D. *Infect. Immun.* 73: 7677–7686.

Lee S.K. and Ch. Josenhans. 2005. *Helicobacter pylori* and the innate immune system. *Int. J. Med. Microbiol.* 295: 325–334.

Lepper P.M., M. Triantafilou, Ch. Schumann, E.M. Schneider and K. Triantafilou. 2005. Lipopolysaccharides from *Helicobacter pylori* can act as antaginsts for Toll-like receptor 4. *Cell. Microbiology* 7: 519–528.

Ljungh A., A.P. Moran and T. Wadstrom. 1996. Interaction of bacterial adhesions with extracellular matrix and plasma proteins: pathogenic implications and therapeutic possibilities. *FEMS Immunol. Med. Microbiol*. 16: 117–126.

Mahdavi J., B. Sonden, M. Hurtig, F.O. Olfat, L. Forsberg and N. Roche. 2002. *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Science* 297: 573–578.

Medvedev A.E., K.M. Kopydlowski and S.N. Vogel. 2000. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and Toll-like receptor 2 and 4 gene expression. *J. Immunol*. 164: 5564–5574.

Moran A.P. and G.O. Aspinall. 1998. Unique structural and biological features of *Helicobacter pylori* lipopolysaccharides. *Prog. Clin. Biol. Res.* 397: 37–49.

Muotiala A., I.M. Helander, L. Pyhala, T.U. Kosunen and A.P. Moran. 1992. Low biological activity of *Helicobacter pylori* lipopolysaccharide. *Infect. Immun*. 60: 1714–1716.

Neumann M., A. Foryst-Ludwig, S. Klor, K. Schweitzer and M. Naumann. 2006. The PAK1 autoregulatory domain is required for interaction with NIK in *Helicobacter pylori*-induced NF-kappa B activation. *Biol. Chem*. 387: 79–86.

Nomura F., S. Akashi, Y. Sakao, S. Sato, T. Kawai, M. Matsumoto, K. Nakanishi, M. Kimoto, K. Miyake, K. Takeda and others. 2000. Endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface Toll-like receptor 4 expression. *J. Immunol.* 164: 3476–3479.

Prohinar P., F. Re, R. Widstrom, Zhang DeSheng, A. Teghanemt, J.P. Weiss and T. Gioannini. 2007. Specific high affinity interactions of monomeric endotoxin protein complexes with Toll-like receptor 4 ectodomain. *J. Biol. Chem*. 282: 1010–1017.

Rechciński T., M. Chmiela, E. Malecka-Panas, I. Planeta-Malecka and W. Rudnicka. 1997. Serological indicators of *Helicobacter pylori* infections in adult dyspeptic patients and healthy blood donors. *Microbiol. Immunol*. 41: 387–393.

Romarao N., S.D. Gray-Owen, S. Backert and T.F. Meyer. 2000. *Helicobacter pylori* inhibits phagocytosis by proffesional phagocytes involving type IV secretion components. *Mol. Microbiol.* 37: 1389–1404.

Rudnicka W., E. Czkwianianc, I. Planeta-Malecka, M. Jurkiewicz, M. Wisniewska, T. Cieslikowski, B. Rozalska, T. Wadstrom and M. Chmiela. 2001. A potential double role of anti-Lewis X antibodies in *Helicobacter pylori*-associated gastroduodenal diseases. *FEMS Immunol. Med. Microbiol.* 30: 121–125.

Smith M.F.Jr., A. Mitchell, G. Li, S. Ding, A.M. Fitzmaurice, K. Ryan, S. Crowe and J. B. Goldberg. 2003. Toll-like receptor TLR2 and TLR5 but not TLR4, are required for *Helicobacter pylori*-induced NF-kappa B activation and chemokine expression by epithelial cells. *J. Biol. Chem.* 278: 32552–32560.

Strapagiel D., A. Grębowska, B. Różalska, L. Bąk-Romaniszyn, E. Czkwianianc, I. Płaneta-Małecka., T. Rechciński, W. Rudnicka and M. Chmiela. 2006. Natural mannose-binding lectin (MBL) down-regulates phagocytosis of *Helicobacter pylori*. *Pol. J. Microbiol*. 55: 95–101.

Suganuma M., T. Kuzuhara, K. Yamaguchi and H. Fujiki. 2006. Carcinogenic role of tumor necrosis factor-alpha inducing protein in human stomach. *J. Biochem. Mol. Biol*. 39: 1–8.

Turina M., F.N. Miller, P.P. McHugh, W.G. Cheadle and H.C. Polk Jr. 2005. Endotoxin inhibits apoptosis but induces primary necrosis in neutrophils. *Inflammation* 29: 55–63.

Underhill D.M. and B. Gantner. 2004. Integration of Toll-like receptor and phagocytic signaling for tailored immunity. *Microbes Infect.* 6: 1368–1373.

Valkonen K. M., T. Wadstrom and A. P. Moran. 1994. Interaction of lipopolysaccharides of *Helicobacter pylori* with the basement membrane protein laminin. *Infect. Immun.* 62: 3640–3648.

Vesy C. J., R. L. Kitchens., G. Wolfbauer., J. J. Alberts and R.S. Munford. 2000. Lipopolysaccharide recognition: CD14, TLRs and LPS-activating cluster. *Trends Immunol*. 23: 301–304.

Wang G., R.C. Conover, A.A. Olaczek, P. Alamuri, M.K. Johnson and R.J. Maier. 2005. Oxidative stress defense mechanisms to counter iron-promoted DNA damage in *Helicobacter pylori*. *Free Radic. Res*. 39: 1183–1191.

Waltz A., S. Odenbreit, J. Mahdavi, T. Boren and S. Ruhl. 2005. Identification and characterization of binding properties of *Helicobacter pylori* by glycoconjugate arrays. *Glycobiology* 15: 700–708.

Wurfel M.M., E. Hailman and S.D. Wright. 1995. Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. *J. Exp. Med.* 181: 1743–1754.

Yu B. and S.D. Wright. 1996. Catalytic properties of lipopolysaccharide (LPS) binding protein. Transfer of LPS to soluble CD14. *J. Biol. Chem.* 271: 4100–4105.

Zweiger J., R. R. Schumann and J. R. Weber. 2006. The role of lipopolysaccharide-binding protein in modulating the innate immune response. *Microbes Infect.* 8: 946–952.