Polish Journal of Microbiology
2006, Vol. 55, No 1, 25–30

Potential Role of LPS in the Outcome of *Helicobacter pylori* Related Diseases

ANETA GRĘBOWSKA[1], TOMASZ RECHCIŃSKI[2], LEOKADIA BĄK-ROMANISZYN[3], ELŻBIETA CZKWIANIANC[3], ANTHONY MORAN[4], MAGDALENA DRUSZCZYŃSKA[1], MAGDALENA KOWALEWICZ-KULBAT[1], AGNIESZKA OWCZAREK[1], MICHAŁ DZIUBA[2], MARIA KRZEMIŃSKA-PAKUŁA[2], IZABELA PŁANETA-MAŁECKA[3], WIESŁAWA RUDNICKA[1] and MAGDALENA CHMIELA[1*]

[1] Department of Immunology and Infectious Biology, University of Łódź, 90-237 Łódź, Banacha 12/16, Poland
[2] II Cardiology Clinic, Medical University in Łódź, 91-347 Łódź, Kniaziewicza 1/5, Poland
[3] Mother Health Center Institute, 93-338 Łódź, Rzgowska 281, Poland
[4] National University of Ireland, Galway, Ireland

Received 16 September 2005, received in revised form 20 December 2005, accepted 21 December 2005

This article is devoted to the memory of the late Prof. W.J.H. Kunicki-Goldfinger on the tenth anniversary of his passing away

Abstract

In this study we asked a question whether *H. pylori* LPS with or without LewisXY (Le) determinants as well as LBP (lipopolysaccharide binding protein) and sCD14 molecules recognizing bacterial LPS may be involved in atherogenesis. Sera from 57 patients with coronary heart disease (CHD), 27 *H. pylori* infected dyspeptic patients-H.p.(+) and 49 healthy controls (HC) were tested for IgM and IgG to *H. pylori* LPS expressing LeX (LPS LeX) or LeXY (LPS LeXY) determinants and to a glycine acid extract (GE). Immune complexes (ICs) of Lewis antigens and specific IgM or IgG were also determined. The prevalence of anti-GE IgG and IgA was significantly higher in CHD as compared to HC and the same as in the H.p.(+) group. The highest levels of anti-GE IgG were detected only for CHD group. CHD patients showed upregulation of IgG to LPS LeX and LeXY. In contrast, an upregulation of IgM to such LPSs was found for healthy subjects. The levels of LeY-anti-LeY IgG ICs were higher in CHD patients than in healthy controls similarly to the levels of LBP. There was no difference in sCD14 concentration between CHD and HC groups. The results obtained in this study indicate that *H. pylori* infections may be the risk factors of atherosclerosis due to: 1) an enhanced humoral response to *H. pylori* surface antigens, 2) a host predisposition to respond to Lewis determinants present in *H. pylori* LPS by IgG, 3) increased levels of serum LBP.

Key words: *Helicobacter pylori*, LPS, atherosclerosis

Introduction

The correlation between *H. pylori* infections and gastro-duodenal diseases was proved in 1983 (Warren and Marshall, 1983). In recent years a link between such infection and coronary heart disease (CHD) has been suggested (Mendall *et al*., 1994). However, in some studies, there was no correlation shown between *H. pylori* and CHD (Koenig *et al*., 1999). In our previous study we showed that both *H. pylori* and *Chlamydia pneumoniae* infections associated with a strong humoral response against such microbes were correlated with CHD (Chmiela *et al*., 2003). Both in gastro-duodenal ulcer diseases and in CHD the inflammatory response induces the pathological processes (Libby, 2002). The soluble (urease, vacuolating cytotoxin-vacA)

* Correspondence to: prof. dr hab. Magdalena Chmiela, Dept. of Immunol. and Infectious Biol., University of Łódź, ul. Banacha 12/16, 90-237 Łódź, Poland, tel: (48 42) 6354472, fax: (48 42) 6655818, e-mail:chmiela@ biol.uni.lodz.pl

and cellular (cytotoxin associated gene A antigen – CagA) *H. pylori* compounds initiate the inflammation in gastric epithelium (Crabtree, 1993). Although LPS is an important proinflammatory compound of Gram-negative bacteria (Alexander and Rietschel, 2001), the structure of lipid A of *H. pylori* probably evolved in the mode which promoted persistence of the infection. The *H. pylori* LPS may regulate the expression of adhesins and it can diminish the secretion of inflammatory cytokines by the host cells (Moutiala *et al*., 1992). Rudnicka *et al*. (2003) showed negative correlation between *H. pylori* LPS driven proliferation of mononuclear leukocytes isolated from dyspeptic patients, and type B inflammation. If so, it is possible that *H. pylori* LPS through the activation of immunocompetent cells may diminish the number of bacteria in gastric tissue and thus prolong the infection. The activity of *H. pylori* LPS is also determined by sugar residues in the O-specific chains, which are similar to Lewis (Le) determinants of the host. *H. pylori* strains may express either LeX or LeY, both or very little of either. The molecular mimicry between bacterial and host determinants may induce the production of autodestructive autoantibodies (Appelmelk *et al*., 1996). The interactions of LPS with the host cells are mediated by cellular and soluble receptors: 1) serum lipopolysaccharide binding protein – LBP; 2) membrane CD14 receptor; 3) sCD14 protein present in circulation which is a soluble form of CD14 receptor; 4) Toll-like receptors (TLR), mainly TLR4, used for signaling and induction of cytokine production in response to LPS (Brightbill and Modlin, 2000; Krutzki *et al*., 2001; Miller *et al*., 2005).

In this study, we asked whether *H. pylori* LPS with or without LeXY determinants as well as LBP and sCD14 molecules may be involved in atherogenesis.

Experimental

Material and Methods

Subjects. One hundred and thirty three individuals aged 30–70 (mean age 59) were included into this study. The first group consisted of 57 patients with coronary heart disease (CHD), hospitalized in the Cardiology Clinic due to a chest pain. Coronary angiography confirmed the atheromatic background of symptoms. In this group, the prevalence of previous myocardial infarction was 52%, arterial hypertension 72%, diabetes mellitus 18%, hyperlipidemia 62%, nicotinism 18%, rate of the revascularization (percutaneous transluminal coronary angiography or coronary artery by-pass graft) – 58%. All patients in this group had a negative history of dyspepsia at least during the last 24 months. The second group consisted of 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 biopsy specimens. All patients in this group had a negative history of cardiovascular symptoms. The healthy control group (HC) included 49 volunteers who had a negative history of cardiovascular and gastric diseases. In this group the prevalence of diabetes mellitus was 3%, arterial hypertension – 13%, hypercholesterolaemia – 12% and nicotinism – 33%. The study was approved by the local Ethical Committee. All participants signed informed consent.

Serological study. Blood from antecubital vein was obtained from all study participants for serological tests. The sera were stored at – 70°C. The Enzyme Linked Immunosorbent Assays – ELISA, were used for estimation:

1) Anti-*H. pylori* IgG and IgA. The ELISA with a glycine acid extract (GE) from the reference *H. pylori* strain CCUG 17874 (Culture Collection University of Gothenburg, Sweden) and rabbit anti-human IgG or IgA antibodies labeled with horseradish peroxidase – HRP (Dako, Glostrup, Denmark) were used according to Rechciński *et al*. (1997). The plates were coated with 5 μg/ml of GE (18 h, 4°C). The serum samples for anti-GE IgG were diluted from 1:500 to 1:128000 and for anti-GE IgA from 1:100 to 1:6400. The results were expressed as total optical density units (OD) measured at 450 nm. The ELISA cutoff, was defined as two standard deviations above the mean OD of control negative sera from the subjects not infected with *H. pylori*.

2) IgG and IgM to *H. pylori* LPS. The ELISA plates were coated with *H. pylori* LPS of Lewis X (LeX) or Lewis XY (LeXY) type (2 μg/ml in 0.15 M phosphate buffered saline-PBS, pH 7.2, 18 h, 20ºC), donated by Dr A. Moran, National University of Ireland, Galway, Ireland. After blocking (1% bovine serum albumin in PBS with 0.05 Tween 80 – BSA/Tween, 2 h at 20°C) the plates were incubated with rabbit HRP anti-human IgG (1:6000) or IgM (1:100) antibodies. The results were expressed as OD450 for the sera diluted 1:100. In every ELISA the controls were included for the elimination of unspecific reactions. The cutoff was defined as double OD for the wells with HRP labeled secondary antibody control.

3) Immune complexes (ICs). ICs LeX-anti-LeX IgG, LeX-anti-LeX IgM, LeY-anti-LeY IgG and LeY-anti-LeY IgM were estimated by ELISA using mouse monoclonal anti-LeX or anti-LeY capture antibodies (Seikagaku, Tokyo, Japan; 100 ng/ml in 0.05 M carbonate buffer, pH 9.6, 18 h at + 4°C). After blocking, the plates were incubated for 1 h, at 37°C with serum samples (1:20) depleted in rheumathoidal factor (Catty and Raykundalia, 1989) and then for the same time with rabbit HRP anti-human IgG (1:6000) or IgM (1:1000) (Dako). The level of specific ICs was expressed as OD450 for the serum samples diluted 1:20. In every ELISA the control wells were included for the elimination of unspecific reactions. The cutoff was defined as double OD450 for HRP labeled secondary antibody control.

4) The serum LBP and sCD14. The commercial ELISA kits were used as recommended by the manufacturer (HyCult Biotechnology, Uden, The Netherlands).

Statistical analysis. 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 comparison of the prevalence of analyzed parameters in studied groups and Spearman's correlation coefficient.

Results

Distribution and the levels of anti-*H. pylori* antibodies. The prevalence of anti-GE IgG was similar in CHD (53/57, 92%) and dyspeptic H.p. (+) patients (27/27, 100%), and significantly lower than in healthy controls (24/49, 49%), $p<0.05$. Similarly, anti-GE IgA were detected in 52 out of 57 CHD patients (90%), in 18 out of 27 dyspeptic patients (67%), and in 12 out of 49 healthy individuals (24%). The total OD450 for anti-GE IgG and IgA was significantly increased in the sera from CHD (5.865 ± 3.665 and 1.754 ± 1.169, respectively) as compared with dyspeptic patients (3.572 ± 1.916 and 0.962 ± 0.505, respectively) and healthy subjects (3.229 ± 1.916 and 0.962 ± 0.505, respectively), $p<0.05$.

The levels of antibodies to *H. pylori* LPS of LeX or LeXY type. The IgG and IgM recognizing *H. pylori* LPS with LeX or LeXY determinants were detected in the sera of all study participants. However, the level of IgG against LPS LeXY type in CHD group was significantly higher than in healthy individuals (Table I). In CHD patients there was an upregulation of the production of IgG to LPS with both LeX and LeXY determinants. In contrast, an upregulation of the production of IgM to such LPSs was found for healthy subjects, $p<0.05$.

Immune complexes (ICs). The ICs LeX-anti-LeX IgG and LeY-anti-LeY IgG were detected for all CHD patients and healthy controls (100%) (Table II). By comparison the ICs LeX-anti-LeX IgM were found in 73% CHD patients and in 90% healthy individuals. Similarly, ICs LeY-anti-LeY IgM were detected in 90% CHD and 93% healthy controls, respectively. The levels of LeY-anti-LeY IgG ICs were higher in CHD patients than in healthy subjects ($p<0.05$). Moreover, the high levels (OD450 > 0.6) of such ICs were detected only for the producers of IgG to GE of *H. pylori*.

Table I
The level of IgG and IgM antibodies against *H. pylori* LPS of LeX or LeXY type in the patients with coronary heart disease – CHD, and healthy controls – HC

Group	The level of anti-*H. pylori* LPS antibodies (OD_{450} 1:100)	
Group	IgG anti-LPS LeXY	IgM anti-LPS LeXY
CHD	0.783 ± 0.450 ↔	0.438 ± 0.204
HC	0.856 ± 0.442 ↔	1.157 ± 0.361
Group	IgG anti-LPS LeXY	IgM anti-LPS LeXY
CHD	↑ 0.891 ± 0.356 ↔	0.650 ± 0.279
HC	↓ 0.719 ± 0.381 ↔	0.835 ± 0.279

↔ difference statistically significant ($p<0.05$)

Table II
The level of immune complexes (ICs) LeX-anti-LeX IgG, LeX-anti-LeX IgM, LeY-anti-LeY IgG and LeY-anti-LeY IgM in the patients with coronary heart disease – CHD and healthy controls – HC

Group	Immune complexes (OD_{450} 1:20)			
	LeX-anti-LeX IgG	LeX-anti-LeX IgM	LeY-anti-LeY IgG	LeY-anti-LeY IgM
CHD	0.372 ± 0.191	0.576 ± 0.192	0.501 ± 0.105 ↑	0.467 ± 0.165
HC	0.328 ± 0.143	0.568 ± 0.179	0.380 ± 0.070 ↓	0.469 ± 0.126
Group	The prevalence of high level of ICs (OD_{450} 1:20>0.6)			
CHD	17%	35%	52% ↑	17%
HC	10%	20%	3% ↓	10%

↔ difference statistically significant ($p<0.05$)

Table III
The level of LBP and sCD14 in the patients with coronary heart disease – CHD and healthy controls – HC

Group	The LBP concentration/prevalence		
	0–10 μg/ml	>10 μg/ml	medium level (μg/ml)
CHD	25/57 (44%)	32/57 (56%) ↑	17.321 ± 16.705 ↑
HC	38/49 (78%)	11/49 (22%) ↓	8.517 ± 4.403 ↓
Group	The sCD14 concentration/prevalence		
	0–4 μg/ml	>4 μg/ml	medium level (μg/ml)
CHD	23/57 (40%)	34/57 (60%)	4.125 ± 2.005
HC	26/49 (53%)	23/49 (47%)	3.808 ± 2.105

↔ difference statistically significant ($p<0.05$)

The serum LBP and sCD14 concentrations. The level of LBP was significantly higher ($p<0.05$) in CHD group (17.321 ± 16.705 μg/ml) as compared with healthy controls (8.517 ± 4.003 μg/ml) (Table III). Similarly, the high level of LBP (>10 μg/ml) was detected more frequently in the sera from CHD patients (56%) than in HC group (22%). Although LBP level was significantly lower in 53 producers of anti-GE IgG (14.911 ± 13.213 μg/ml) than in 4 seronegative CHD patients (49.207 ± 27.801 μg/ml), there was no difference in LBP concentration in the seropositive or seronegative healthy controls. In CHD patients and healthy individuals the level of sCD14 was similar, 4.125 ± 2.050 μg/ml and 3.808 ± 2.115 μg/ml, respectively (Table III). The sCD14 concentration over 4 mg/ml was detected more frequently in CHD patients as compared with healthy individuals, 60% and 40% respectively. However, the difference was not significant.

Discussion

It has been suggested that *H. pylori* infection can be involved in atherogenesis (Mendall, 1994). In this study the prevalence of anti-GE IgG and IgA was significantly higher in CHD patients than in healthy subjects and the same as in H.p. (+) dyspeptic patients. Also the levels of anti-GE IgG/IgA were higher in CHD than in healthy group. It is possible that gastrotoxic activity of aspirin used by the CHD patients for a long time may facilitate the penetration of bacterial antigens through the mucosal barrier and a strong humoral response to them (Sims *et al*., 2000).

The evolution has led to co-expression of common LeX and LeY carbohydrates which are present in the LPSs of most *H. pylori* strains and in human cell surface glycoconjugates of blood cells and gastric mucosa. Based on such molecular mimicry Appelmelk *et al*. (1996) suggested the role of autoimmune mechanisms in *H. pylori* associated type B gastritis. Recently *H. pylori* CagA has been considered as putative autoimmune antigen (Takahashi *et al*., 2004).

An upregulation of IgG and IgA to *H. pylori* antigens in CHD patients prompted us to ask whether LeX and LeY determinants of *H. pylori* LPS could be involved in atherogenesis. There was no difference between CHD and healthy individuals with regard to the prevalence of IgG and IgM to *H. pylori* LPS of LeX or LeXY type. However, the level of IgG to *H. pylori* LPS LeXY was significantly increased in CHD patients as compared with healthy subjects. Moreover, the CHD patients showed an upregulation of the production of IgG to LPS of LeX or LeXY type. In contrast, an upregulation of IgM production to LPS with LeX or LeXY was observed in healthy subjects. Cedzyński *et al*. (1998) showed that during natural history of *H. pylori* infections in humans, mainly antibodies of IgG class to polysaccharide chains of LPS of these bacteria are produced. The presence of antibodies to *H. pylori* LPS LeX/Y in healthy subjects seronegative to anti-GE IgG could be due to the reactivity to Lewis epitopes expressed on some unrelated microbes (Hirota *et al*., 1995) or it could result from an immunological response to self LeX or LeY antigens. Amano *et al*. (1995) observed the reaction of human sera with the synthetic Le antigens regardless of the status of the individual's *H. pylori* infection. We cannot exclude that inflammatory and/or physiological abnormalities leading to chronic dyspepsia or atherosclerosis should be considered as signals for the production of anti-LeX/Y antibodies. The presence of such antibodies in the sera from healthy individuals with negative *H. pylori* serology also implies that the production of anti-LeX/Y IgG and particularly IgM might be a physiological state. Such Ig's, similarly to anti-Le a or anti-Le b antibodies occur naturally (Henry *et al*., 1995). Under the influence of genetic or environmental factors, including *H. pylori* infection, this normal response to self-antigens may develop into autoimmune disease and contribute to tissue damage.

In a self-destructive autoimmune response an important role is played by antigen-antibody ICs. In this study the levels of LeY-anti-LeY IgG ICs were higher in CHD patients than in healthy controls. The highest levels of ICs were detected only for the producers of IgG to *H. pylori* GE. Sims *et al*. (2000) reported that in the vessels of the patients with atherosclerosis the ICs were deposited. Our results suggest that during chronic *H. pylori* infections a host predisposition to respond to Le determinants by IgG but not IgM could be one of the risk factors for atherogenesis. The ICs formed of Le antigens and specific IgG may persist in a circulation as the small ones longer than the big ones formed of antigen and IgM. The enhanced blood pressure and turbulences of circulation in small arteries may promote in CHD patients a deposition of ICs. This may stimulate secretion of proinflammatory cytokines by the macrophages and granulocytes *via* FcγR receptors. The activation may be followed by the release of lysosomal enzymes, cationic proteins, reactive oxygen and nitrogen intermediates which promote tissue injury (Clynes *et al*., 1999). Anti-LeX/Y antibodies may enhance in *H. pylori* infected subjects the inflammatory effect of bacterial CagA, VacA and urease. The long term inflammation generated by *H. pylori* may raise cytokine levels in the bloodstream, and activate

fibroblast and smooth muscle cell proliferation which is an important step in atherogenic process. In *H. pylori* infected subjects a stimulation of T lymphocytes by bacterial LPS is very likely. Activated lymphocytes may facilitate a control of bacterial growth and diminish inflammation in gastric mucosa by releasing cytokines, possibly of Th2 type. Weak inflammatory response may help the bacteria to survive in the host tissue (Rudnicka and Chmiela 2004).

Bacterial LPS bound with LBP is delivered to membrane or soluble CD14 molecules (mCD14 and sCD14, respectively) and, therefore initiates TLR4 signaling (Duzendorfen *et al*., 2004). Such TLRs are expressed on macrophages, granulocytes and dendritic cells but also on endothelial cells and macrophages in atherosclerotic plaques (Van Haelst *et al*., 2004). In this study significantly higher levels of LBP were observed in CHD patients as compared with healthy subjects. The involvement of *H. pylori* infection in CHD may result in the enhancement of LBP production and possible deposition of lipid-LBP and/or LPS-LBP complexes in endothelium. The linkage of *H. pylori* infections with increased levels of total cholesterol and triglicerides has been found (Niemela *et al*., 1996; Schanagl *et al*., 2003). Eilersten *et al*. (2003) showed a correlation between CD14 –159 C/T polymorphism and the ability of CD14 to bind cholesterol. The TT homozygotes were associated with lower total cholesterol, LDL and apolipoprotein B-100 concentrations. Our preliminary results show that CT genotype was more frequently detected in the patients with myocardial infarction as compared with unstable angina pectoris group. Edfeldt *et al*. (2004) identified susceptibility to myocardial infarction in men carrying both TLR4 299Gly and 399Ile allele. An increased risk of atherosclerosis is also associated with RANTES G-40A gene polymorphism (Simeoni *et al*., 2004). Grębowska *et al*.(2004) reported that Le determinants of *H. pylori* LPS modulate the CD14 mediated cytokine response of macrophages. The infection can also predispose to atherosclerosis by an endothelial dysfunction (Prosad *et al*., 2002). In conclusion, the results obtained in this study: 1) the enhanced humoral response to *H. pylori* antigens in the patients with coronary heart disease, 2) the host predisposition to respond to LeXY determinants of *H. pylori* LPS by IgG but not IgM, and 3) the increased levels of serum LBP, indicate that *H. pylori* infections may be recognized as the risk factors of atherosclerosis.

Literature

Alexander Ch. and E.T. Rietschel. 2001. Bacterial lipopolysaccharides and innate immunity. *J. Endotox. Res.* **7**: 167–2002.

Amano K.I., S. Kayashi and T. Kubota. 1995. Reactivities of Lewis antigen monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains from patients with gastroduodenal diseases in Japan. *Clin. Diagn. Lab. Immunol*. **4**: 540–544.

Appelmelk B.J, R. Simmoons-Smit, R. Negrini, A.P. Moran, G.O. Aspinall, J.G. Forte, T. de Vreis, H. Quan, T. Verboom, J.J. Maaskant, P. Ghiara, E.J. Kuipers, E. Bloemena, T.M. Tadema, R.R. Townsend, K. Tyagarajan, J.M. Jr. Crothers, H.A. Monteiro, A. Sario and J. Graaff. 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. *Infect. Immun*. **64**: 2031–2040.

Brightbill H.D. and R.L. Modlin. 2000. Toll like receptors: molecular mechanisms of the mammalian immune response. *Immunology* **101**: 1–10.

Catty D. and C. Raykundalia. 1989. ELISA and related enzyme immunoassays, in: Catty, D. (Ed.), Antibodies volume II a practical approach. IRL Press, Oxford University Press, pp. 97–152.

Cedzyński M., K.I. Amano, M. Chmiela, M. Ławnik, E. Czkwianianc, I. Płaneta-Małecka, W. Rudnicka and W. Kaca. 1998. Serum IgG antibodies in children and adults, reacting with *Helicobacter pylori* lipopolysaccharides. *Arch. Immunol. Ther. Exp*. **46**: 79–83.

Chmiela M., M. Kowalewicz-Kulbat, A. Miszczak, M. Wiśniewska, T. Rechciński, K. Kołodziej, J. Kasprzak, T. Wadstrom and W. Rudnicka. 2003. A link between *Helicobacter pylori* and/or *Chlamydia* spp. Infections and atherosclerosis. *FEMS Immunol. Med. Microbiol*. **36**: 187–192.

Clynes R., J.S. Mainez, R. Guinamard, M. Ono, T. Takai and J.V. Ravetch. 1999. Modulation of immune-complex induced inflammation *in vivo* by the coordinate expression of activation and inhibitory Fc receptors. *J. Exp. Med*. **189**: 179–185.

Crabtree J.E. 1993. Mucosal immune response to *Helicobacter pylori*. *J. Gastroenterol. Hepatol.* **5**: 30–32.

Duzendorfer S., H.K. Lee, K. Soldau and P.S. Tobias. 2004. TLR4 is the signaling but not the lipopolysaccharide uptake receptor. *J. Immunol*. **173**: 1166–1170.

Edfeldt K., A.M. Bennet, P. Eriksson, J. Frostegard, B. Wiman, A. Hamsten, G.K. Hansson, U. Faire and Z. Yan. 2004. Association of hypo-responsive toll-like receptor 4 variants with risk of myocardial infarction. *Eur. Heart J*. **25**: 1447–1453.

Eilersten K.E., J.O. Olsen, J. Brox and B. Osterud. 2003. Association of the –159→T polymorphism in the CD14 promoter with variations in serum lipoproteins in healthy subjects. *Blood Coag. Fibrinol*. **14**: 663–670.

Grębowska A., A. Moran, E. Czkwianianc, L. Bąk-Romaniszyn, I. Płaneta-Małecka, W. Rudnicka and M. Chmiela. 2004. A modulation of macrophage response to *H. pylori* LPS with or without Lewis XY determinants. *Helicobacter* **9**: 05.16, 523.

Henry S., R. Orioland and B. Samuelsson. 1995. Lewis histo-blood group system and associated secretory phenotypes. *Vox Sang.* **69**: 166–182.

Hirota K., H. Kanitani, K. Nemoto, T. Ono and Y. Miyake. 1995. Crossreactivity between human sialyl Lewis X oligosaccharide and common causative oral bacteria of infective endocarditis. *FEMS Immunol. Med. Microbiol*. **12**: 159–164.

Koenig W., D. Rothenbacher, A. Hoffmister, M. Miller, G. Bode, G. Adler, V. Hombach, W. Marz, M.B. Pepys and H. Brenner. 1999. Infection with *Helicobacter pylori* is not a major independent risk factor for stable coronary heart disease. Lack of role of cytotoxin-associated protein A positive strains and absence of a systemic inflammatory response. *Circulation* **100**: 2326–2331.

Krutzik S., P.A. Sieling and R.L. Modlin. 2001. The role of Toll-like receptors in host defense against microbial infections. *Curr. Opin. Immunol*. **13**: 104–108.

Libby P. 2002. Inflammation in atherosclerosis. *Nature* **420**: 868–874.

Mendall M., P. Goggin, M. Molineaux, Y. Levy, T. Toosy, D. Shacham, A.J. Camm and T.C. Northfled. 1994. Relation of *Helicobacter pylori* infection and coronary heart disease. *Br. Heart J*. **71**: 437–439.

Miller S.I., R.K. Ernst and M.W. Bader. 2005. LPS, TLR4 and infectious disease diversity. *Nature Rev. Microbiol*. **3**: 36–46.

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

Niemela S., T. Karttunen, T. Korbonen, E. Laara, R. Karttunen, M. Ikaheimo and Y.A. Kesaniemi. 1996. Could *Helicobacter pylori* infection increase the risk of coronary heart disease by modifying serum lipid concentrations? *Heart* **75**: 573–575.

Prosad A., J. Zhu, J.P.J. Halcox, M.A. Waclawin, S.E. Epstein and A.A. Quyyumi. 2002. Predisposition of atherosclerosis by infections. Role of endothelial dysfunction. *Circulation* **106**: 184–190.

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

Rudnicka W., A. Jarosińska, L. Bąk-Romaniszyn, A. Moran, I. Płaneta-Małecka, T. Wadstrom and M. Chmiela. 2003. *Helicobacter pylori* lipopolysaccharide in the IL-2 milieu activates lymphocytes from dyspeptic children. *FEMS Immunol. Med. Microbiol*. **36**: 141–145.

Rudnicka W. and M. Chmiela. 2004. Inflammation and host immune response in *Helicobacter pylori* infections. *Curr. Trends Immunol*. **6**: 1–19.

Schanagl H., M. Kist, A.B. Grawitz, W. Koenig, H. Wieland and W. Maerz. 2003. *Helicobacter pylori* eradication increases high density lipoproteins. *Atherosclerosis Suppl.* (*ATHSUP*) **4**: 73.

Simeoni E., B.R. Winkelmann, M.M. Hoffman, S. Fleury, J. Ruiz, L. Kappenberger, W. Marz and G. Vassali. 2004. Association of RANTES G-403A gene polymorphism with increased risk of coronary arteriosclerosis. *Eur. Haert J*. **25**: 1438–1446.

Sims F.H., J.B. Gavin, S. Edgar and T. Koelmeyer. 2000. Diffusion of gammaglobulin into the arterial wall identifies localized entry of lipid and cells in atherosclerosis. *Coronary Art. Dis*. **12**: 21–30.

Takahashi T., T. Yujiri, T., K. Shinohara, Y. Inoue, Y. Sato, Y. Fuji, M. Okubo, Y. Zaitsu, K. Ariyoshi, Y. Nakamura, R. Nawata, Y. Oka, M. Shiraiand and Y. Tanizawa. 2004. Molecular mimicry by *Helicobacter pylori* CagA protein may be involved in the pathogenesis of *H. pylori*-associated chronic idiopathic thrombocytopenic purpura. *Br. J. Haematol*. **124**: 91–96.

Van Haelst P.L., T.J. Cohen, J. Bijzet, C. Balje-Volkers, J.F. May, B. Langeveld and R. Gans. 2004. Circulating monocytes in patients with acute coronary syndromes lack sufficient interleukin-10 production after lipopolysaccharide stimulation. *Clin. Exp. Immunol*. **138**: 364–368.

Warren J.R. and B.J. Marshall. 1983. Unidentified curved bacilli on the gastric epithelium in active chronic gastritis. *Lancet* **1**: 1237–1275.