PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(14):2055-2059
World Journal of Gastroenterology
Copyright ©2004 by The WJG Press ISSN 1007-9327

Helicobacter pylori lipopolysaccharide: Biological activities *in vitro* and *in vivo*, pathological correlation to human chronic gastritis and peptic ulcer

Yi-Hui Luo, Jie Yan, Ya-Fei Mao

Yi-Hui Luo, Jie Yan, Ya-Fei Mao, Department of Medical Microbiology and Parasitology, College of Medical Science, Zhejiang University, Hangzhou 310031, Zhejiang Province, China
Supported by the Foundation of Ministry of Education of China for Distinguished Young Scholars
Correspondence to: Professor Jie Yan, Department of Medical Microbiology and Parasitology, College of Medical Science, Zhejiang University, 353 Yan An Road, Hangzhou 310031, Zhejiang Province, China. yanchen@mail.hz.zj.cn
Telephone: +86-571-87217385 **Fax:** +86-571-87217044
Received: 2003-12-23 **Accepted:** 2004-01-12

Abstract

AIM: To determine the biological activity of *Helicobacter pylori* (*H pylori*) lipopolysaccharide (H-LPS) and understand pathological correlation between H-LPS and human chronic gastritis and peptic ulcer.

METHODS: H-LPS of a clinical *H pylori* strain and LPS of *Escherichia coli* strain O55:B5 (E-LPS) were extracted by phenol-water method. Biological activities of H-LPS and E-LPS were detected by limulus lysate assay, pyrogen assay, blood pressure test and PBMC induction test in rabbits, cytotoxicity test in NIH 3T3 fibroblast cells and lethality test in NIH mice. By using self-prepared rabbit anti-H-LPS serum as the first antibody and commercial HRP-labeled sheep anti-rabbit sera as the second antibody, H-LPS in biopsy specimens from 126 patients with chronic gastritis (68 cases) or gastric ulcer (58 cases) were examined by immunohistochemistry.

RESULTS: Fibroblast cytotoxicity and mouse lethality of H-LPS were weaker than those of E-LPS. But the ability of coagulating limulus lysate of the two LPSs was similar (+/0.5 ng/mL). At 0.5 h after H-LPS injection, the blood pressures of the 3 rabbits rapidly declined. At 1.0 h after H-LPS injection, the blood pressures in 2 of the 3 rabbits fell to zero causing death of the 2 animals. For the other one rabbit in the same group, its blood pressure gradually elevated. At 0.5 h after E-LPS injection, the blood pressures of the three rabbits also quickly declined and then maintained at low level for approximately 1.0 h. At 0.5 h after injection with H-LPS or E-LPS, PBMC numbers of the rabbits showed a remarkable increase. The total positivity rate of H-LPS from 126 biopsy specimens was 60.3%(76/126). H-LPS positivity rate in the biopsy specimens from chronic gastritis (50/68, 73.5%) was significantly higher than that from gastric ulcer (26/58, 44.8%) (χ^2=10.77, P<0.01). H-LPS positivity rates in biopsy specimens from chronic superficial gastritis (38/48, 79.2%) and chronic active gastritis (9/10, 90.0%) were significantly higher than that of the patients with atrophic gastritis (3/10, 30.0%) (χ^2=7.50-9.66, P<0.01).

CONCLUSION: The biological activities of H-LPS were weaker than those of E-LPS, the activities of H-LPS of lowering rabbit blood pressure and inducing rabbit PBMC were relatively stronger. H-LPS may play a critical role in inducing inflammatory reaction in human gastritis.

Luo YH, Yan J, Mao YF. *Helicobacter pylori* lipopolysaccharide: Biological activities *in vitro* and *in vivo*, pathological correlation to human chronic gastritis and peptic ulcer. *World J Gastroenterol* 2004; 10(14): 2055-2059
http://www.wjgnet.com/1007-9327/10/2055.asp

INTRODUCTION

Gastritis and peptic ulcer are the most prevalent gastric diseases. Gastric cancer is one of the malignant tumors with high morbidities in China[1]. *Helicobacter pylori* (*H pylori*) is recognized as a human-specific gastric pathogen that colonizes the stomachs of at least half of the world' s populations[2]. Most infected individuals are asymptomatic. However, in some subjects, the infection causes acute, chronic gastritis or peptic ulceration, and plays an important role in the development of peptic ulcer and gastric adenocarcinoma, mucosa-associated lymphoid tissue lymphoma and primary gastric non-Hodgkin' s lymphoma[3-7].

H pylori is a microaerophilic Gram-negative bacillus. It is well known that lipopolysaccharide (LPS) is a common and essential component in outer membrane of most Gram-negative bacteria responsible for the toxicity of endotoxin. Some literatures revealed *H pylori* possesses LPS (H-LPS) with a lower virulence compared to the typical bacterial endotoxins such as *Escherichia coli* LPS (E-LPS)[8-12]. However, some biological activities such as regulating blood pressure and inducing peripheral blood mononuclear cell (PBMC), which are clinically important in local tissue inflammation and injury, and pathological importance of H-LPS in human gastric diseases are still little understood. Besides, some previously published data demonstrated that different strains of the same bacterium and different extraction methods would significantly affect the biological activity of LPS[13,14].

For measurement of the biological activities of H-LPS *in vivo* and *in vitro* compared to a typical LPS from *E. coli*, we used phenol-water method to extract LPS from a clinical isolated *H pylori* strain and applied routine assays for determining the endotoxin activity of H-LPS such as limulus lysate agglutination, rabbit pyrogenicity and mouse lethality. Furthermore, we also examined H-LPS activities on blood pressure regulation and PBMC inducement. To obtain direct evidence for the correlation of H-LPS and human chronic gastritis and peptic ulcer, we detected H-LPS in gastric biopsy specimens from patients with different gastric diseases.

MATERIALS AND METHODS

Bacterial strains and culture

A clinical *H pylori* strain named as Y06 was isolated from a

biopsy specimen of a male patient with chronic superficial gastritis and duodenal ulcer by using selected Columbia agar (bioMérieux) supplemented with 80 mL/L sheep blood, 5 g/L cyclodextrin, 5 mg/L trimethoprime, 10 mg/L vancomycin, 2.5 mg/L amphotericin B and 2 500 U/L cefsulodin. This strain was identified as *H pylori* based on its typical Gram staining morphology, positivity for both urease and oxidase, and agglutination with a commercial rabbit antibody against whole cell of *H pylori* (DAKO). *E. coli* strain O55:B5 was offered by the National Institute for the Control of Pharmaceuticals and Biological Products of China (NICPBP) and cultured with BL agar.

LPS extraction

H-LPS from *H pylori* strain Y06 and E-LPS from *E. coli* strain O55:B5 were extracted by phenol-water method. Briefly, the two bacteria collected from Columbia agar and BL agar were ultrasonically broken, respectively. Each of the broken bacterial solutions was added with an equal volume of pre-warmed phenol-water (9:1, V:V) and then vibrated for 30 min at 68-70 ℃. The aqueous phase was collected after centrifugation at 3 000 r/min for 30 min. This extraction step was repeated for 3 times. All the aqueous phases were combined and then dialyzed against distilled water for 48 h. This rough LPS extract was concentrated to 1/6 of the original volume and then digested with RNase H and DNase I (Sigma) to both the final concentration of 50 μg/mL at 37 ℃ for 4 h. The digested extract was bathed in boiling-water for 15 min and then placed at 4 ℃ overnight. The supernatant obtained after centrifugation at 3 000 r/min for 30 min was dialyzed against distilled water for 48 h and then precipitated with 6-fold volumes of anhydrous alcohol at 4 ℃ for 12 h. The precipitate was collected by centrifugation at 5 000 r/min for 30 min and then re-suspended with distilled water and dialyzed against distilled water for 24 h to remove residual alcohol. The LPS extract was ultra-centrifuged at 110 000 *g* for 3 h (4 ℃) and the pellet was dialysed in distilled water and freeze-dried. The purified H-LPS and E-LPS were dissolved in pyrogen-free water or normal saline prepared with pyrogen-free water just before different uses.

Limulus lysate assay

Limulus lysate assay was applied by using E-TOXATE Reagent Kit (Sigma) to detect the H-LPS and E-LPS preparations according to the manufacturer' s instruction (Sensitivity = +, 1 ng/mL *E. coli* O55:B5 LPS). In this assay, *E. coli* O55:B5 LPS (Sigma) and pyrogen-free water were used as the positive and negative controls, respectively.

Rabbit pyrogen assay

Anal temperatures of normal New Zealand rabbits with 3.0±0.2 kg of body mass were detected for3 times at an interval of 30 min before performing the test. A rabbit was suitable for the test if the fluctuant range of the three detected temperatures was ≤0.2 ℃. The qualified rabbits were randomly divided into three groups and each group contained three animals. Each of the three rabbits in one group was injected with 0.5 mL normal saline containing H-LPS or E-LPS at the same dosage of 100 μg/kg through ear vein. Each of the three rabbits in the 3rd group was injected with an equal volume of pyrogen-free normal saline as a negative control. Anal temperature in each of the tested rabbits was detected at an interval of 30 min for 5 h after injection. The positive standard for rabbit pyrogen assay was described as following: body temperature for any one of the three rabbits in one group showed ≥0.6 ℃ elevation, or the total elevated body temperature for the three rabbits in one group was ≥1.4 ℃.

Rabbit blood pressure regulation test

Normal New Zealand rabbits with 3.0±0.2 kg of body mass were ear-intravenously injected with 0.5 mL pyrogen-free normal saline. Then blood pressures of the animals were observed for three times at an interval of 30 min. A rabbit was suitable for this test if its blood pressure fluctuation was within 0.2 kPa. The qualified rabbits were divided into three groups and each contained three animals. Each of the three rabbits in one group was ear-intravenously injected with pyrogen-free saline containing H-LPS or E-LPS at the same dosage of 100 μg/kg and each of the three rabbits in the 3rd group were ear-intravenously injected with an equal volume of pyrogen-free saline as a negative control. The changes of blood pressure in the tested rabbits were observed.

PBMC inducement test

PBMC numbers in blood from ear vein of normal New Zealand rabbits with 3.0±0.2 kg of body mass were counted with hemacytometer twice at an interval of 30 min before performing the test. These rabbits were randomly divided into three groups and each contained 5 animals. Each of the five rabbits in one group was ear-intravenously injected with pyrogen-free saline containing H-LPS or E-LPS at the same dosage of 100 μg/kg and each of the five rabbits in the 3rd group were ear-intravenously injected with an equal volume of pyrogen-free saline as a negative control. The change of PBMC numbers for each of the tested rabbits was counted at an interval of 30 min after injection.

Cytotoxicity test

Microtiter plates were inoculated with 1×10^4 mouse 3T3 fibroblast cells in RPMI 1640 medium containing 100 mL/L bovine serum per well and then incubated at 37 ℃ overnight in 50 mL/L CO_2 atmosphere. The medium in the plates was discarded and then added with 200 μL of fresh medium containing H-LPS or E-LPS with different double dilutions. For each of the dilutions, 3 wells were repeated and then incubated for 48 h under the same conditions as mentioned above. In this test, the other 5 wells added with the same volume of LPS-free medium were set up as a negative control. ^{3}H-TdR per well (37 kBq) was added and then continuously incubated for 24 h. CPM value for each of the wells was detected by using ^{3}H-TdR incorporation method to compare the cytotoxicity of H-LPS and E-LPS.

Mouse lethality test

NIH mice weighing 20±2 g were randomly divided into 7 groups and each contained 8 animals. For 6 of the 7 groups, each of the mice in one group was intraperitoneally injected with 0.2 mL pyrogen-free normal saline containing H-LPS or E-LPS at the different dosages of 0.25, 0.50 and 1.0 mg, respectively. Each of the mice in the last group was intraperitoneally injected with 0.2 mL pyrogen-free saline as a negative control. All the tested animals were observed for 7 d.

Detection of H-LPS in biopsy specimens from patients with chronic gastritis and gastric ulcer

Biopsy specimens with positive urease from 126 patients (86 male, 40 female, mean age: 40±18 years) with gastric diseases during January to September of 2003 were collected from three hospitals in Hangzhou. Among these patients, 68 suffered from chronic gastritis (48 cases with chronic superficial, 10 active and 10 atrophic gastritis) and 58 cases suffered from gastric ulcer (12 cases with gastric, 40 duodenal and 6 complex ulcer). The biopsy specimens were fixed with 50 g/L glutaraldehyde. Rabbit anti-H-LPS serum was prepared by using routine subcutaneous immunization. By using the

rabbit anti-H-LPS serum (1:200 dilution, self-prepared) as the first antibody and HRP-labeled sheep anti-rabbit serum (1:3 000 dilution, ImmunoResearch) as the second antibody, H-LPS in the biopsy specimens was detected by routine immunohistochemistry. Cells with at least one whole gastric gland in a biopsy section showing exactly brown color could be considered as positive.

RESULTS

Limulus lysate coagulation

The ability of coagulating limulus lysate of H-LPS was as low as 0.5 ng/mL, which was similar to E-LPS (Table 1).

Table 1 Results of limulus lysate assay of H-LPS and E-LPS

Group	LPS (ng/mL)							
	10.0	5.0	2.5	1.0	0.5	0.25	0.1	0.05
H-LPS	+	+	+	+	+	-	-	-
E-LPS	+	+	+	+	+	-	-	-
Pyrogen-free water	negative in two repeated samples							

Pyrogenic response

The rabbits injected with H-LPS or E-LPS showed a similar biphasic fever but E-LPS could induce a stronger pyrogenic response. The animal temperature reached a peak at 1.5 h after injection and then showed a slight fall. At 3 h after injection, the second fever peak occurred (Figure 1).

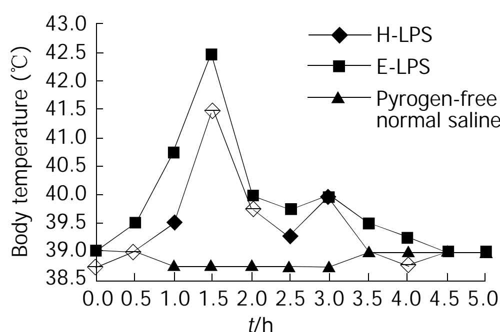

Figure 1 Fever curves of rabbits after injection with H-LPS or E-LPS.

Regulation of rabbit blood pressure

At 0.5 h after H-LPS injection, the blood pressures of the three rabbits rapidly declined from originally 11.331±0.152 kPa to 5.999±1.855 kPa. At 1.0 h after H-LPS injection, the blood pressures in two of the three rabbits fell to zero causing death of the two animals. For the other one rabbit in the same group, its blood pressure gradually elevated. At 0.5 h after E-LPS injection, the blood pressures of the three rabbits also quickly declined from originally 11.197±0.163 kPa to 8.531±2.424 kPa and then maintained the similar low blood pressure levels for approximately 1.0 h. At 2.5 h after injection, the blood pressures in all the three rabbits began to rise and returned to the original levels gradually.

PBMC inducement

At 0.5 h after injection with H-LPS or E-LPS, PBMC numbers of the rabbits showed a remarkable increase. From 1.0 h after injection with H-LPS or E-LPS, the PBMC numbers gradually and continuously decreased to low levels (Table 2).

Cytotoxicity to mouse fibroblast

Very low dosage of H-LPS or E-LPS (1 μg/mL) could show an obvious cytotoxicity to NIH 3T3 fibroblast. According to the counting per minute (CPM) of scintillation at the same concentrations, cytotoxicity of H-LPS seemed to be a little weaker than that of E-LPS (Table 3).

Table 2 PBMC number changes in the rabbits injected with H-LPS or E-LPS

Time (h)	PBMC (mean±SD, ×10⁹/L)		
	H-LPS	E-LPS	Saline
Before injection	4.52±1.28	4.38±1.35	4.05±1.42
After injection			
0.5	12.51±0.54	8.22±0.68	4.25±1.28
1.0	2.55±0.87	2.23±1.32	4.40±1.19
1.5	1.56±1.33	1.58±1.27	4.08±1.24
2.0	1.62±0.93	1.64±0.89	4.05±1.06
2.5	1.65±1.12	1.60±1.34	4.35±1.17
3.0	1.56±1.45	1.52±1.10	4.16±1.22

Table 3 Cytotoxicity of H-LPS and E-LPS to mice fibroblast

Group	LPS (μg/mL)	CPM (mean±SD)	*t*	*P*
H-LPS	1.0	7 722±819	3.74	<0.05
	5.0	4 724±726	7.29	<0.01
	10.0	4 328±1 194	6.56	<0.01
	50.0	3 618±434	9.27	<0.001
	100.0	2 963±764	9.20	<0.001
E-LPS	1.0	6 813±1 183	4.17	<0.05
	5.0	4 290±474	8.37	<0.005
	10.0	3 516±645	8.96	<0.001
	50.0	3 224±534	9.55	<0.001
	100.0	2 513±630	10.72	<0.001
Control	0	11 083±1 324		

Mouse lethality

H-LPS showed a significantly weaker virulence to mice than E-LPS. Although the injecting dosage was as high as 1 mg H-LPS per mouse, 2 of the 8 mice survived (Table 4).

Table 4 Toxicity test results in mice injected with H-LPS or E-LPS

Group	Number (*n*)	Dosage (mg/mouse)	Death/Survival (*n*/*n*)	Mortality (%)
H-LPS	8	0.25	0/8	0
	8	0.50	4/4	50.0
	8	1.00	6/2	75.0
E-LPS	8	0.25	2/6	25.0
	8	0.50	7/1	87.5
	8	1.00	8/0	100
	8	/	0/8	0

Positivity rate of H-LPS in biopsy specimens

The total positivity rate of H-LPS in the 126 biopsy specimens was 60.3% (Table 5). Totally 73.5% of the biopsy specimens from chronic gastritis patients (50/68) were H-LPS positive, which was significantly higher than that (44.8%) from gastric ulcer patients (26/58) (χ^2=10.77, P<0.01). H-LPS positivity rate in biopsy specimens of the patients with chronic superficial gastritis (38/48, 79.2%) was similar to that of the patients with chronic active gastritis (9/10, 90.0%) (χ^2=0.63, P>0.05), but both positivity rates were much higher than that of the patients with atrophic gastritis (3/10, 30.0%) (χ^2=7.50-9.66, P<0.01). Among the biopsy specimens from patients with any one of

the three types of gastric ulcer, the H-LPS positive rates were similar to each other (χ^2=0.11-1.25, P>0.05). A H-LPS positive biopsy specimen is shown in Figure 2.

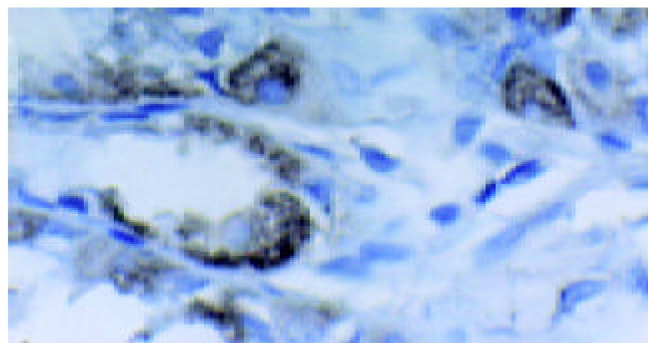

Figure 2 H-LPS positive biopsy specimen (Original magnification: ×600).

Table 5 H-LPS detection rates in the biopsy specimens from patients with chronic gastritis and gastric ulcer

Group	Number (*n*)	Positivity (*n*)	Positive rate (%)
Chronic gastritis			
Superficial	48	38	79.2
Active	10	9	90.0
Atrophic	10	3	30.0
Gastric ulcer			
Gastric	12	7	58.3
Duodenal	40	16	40.0
Complex	6	3	50.0
Total	126	76	60.3

DISCUSSION

Bacterial endotoxin possesses broad biological activities and its toxicity is mainly dependent on lipid A[15]. Among the biological activities of LPS, limulus amoebocyte lysate coagulation, rabbit pyrogen and mouse lethality are most typical and important[16,17]. In some of the previously published data, H-LPS showed much lower activities of coagulating limulus lysate, pyrogenic response in rabbit and lethal potential in mice[8,9]. In our study, we found that the effect of H-LPS on causing fever in rabbits, death in mice and its cytotoxicity to NIH 3T3 fibroblast were less compared to the LPS from *E. coli*. However, the ability of coagulating limulus lysate of H-LPS (+/0.5 ng/mL) was similar to that of E-LPS based on several repeated results. As mentioned in Introduction, LPS preparations might have various biological activities if different extraction methods were used[13,14]. The result in limulus lysate assay of this study differed from the previously reported probably due to H-LPS from different strains and the distinction in LPS extraction methods.

Salgado *et al.* revealed that H-LPS from different strains could be divided into two types: One of low biological activity and one of high biological activity of inducing the mitogenicity and TNF-α synthesis of cells. And the strains with the high activity were demonstrated belonging to the low virulence genotypes with *cagA*$^-$ and s1bm2 or s2m2 for *vacA*[18]. To our surprise, at the same injected dosage (100 μg/kg· b.w.) in our study, H-LPS caused death in two of the three tested rabbits in blood pressure regulation test but E-LPS did not. In addition, the ability of H-LPS of inducing PBMC at 0.5 h after injection (12.51±0.54×10^9/L) was also stronger than that of E-LPS [(8.22±0.68)×10^9/L]. These data, including the results reported by Salgado *et al.*, indicated that the role of the LPS as a virulence factor from some *H pylori* strains should be re-evaluated.

It was reported that H-LPS acted as a modulator of host-dependent gastritis through inducing both gastric epithelial cells and macrophages to secrete IL-1, TNF and IL-8[19-21]. In this study, a high frequency of H-LPS in the biopsy specimens from chronic gastritis patients (73.5%) was found. However, the positivity rate of H-LPS in the biopsy specimens from gastric ulcer patients (44.8%) was relatively lower (χ^2=10.77, P<0.01). Furthermore, the biopsy specimens from chronic superficial gastritis (79.2%) and chronic active gastritis (90.0%) showed significantly higher H-LPS positive rates compared to those from chronic atrophic gastritis (30.0%) (χ^2=7.50-9.66, P<0.01). These data indicated that H-LPS might play a more important role in inducing human gastric inflammation than previously considered. It is well known that PBMC are a mixture of neutrophilic granulocytes, mononuclear macrophages and lymphocytes[22-24]. Mononuclear macrophages and lymphocytes are the major cells to produce IL-1, TNF and IL-8[25-27]. The result from our study showed that H-LPS had a stronger ability of inducing PBMC than E-LPS, suggesting the critical effect of H-LPS on inducing inflammatory reaction in human gastritis.

REFERENCES

1 **Zhang Z**, Yuan Y, Gao H, Dong M, Wang L, Gong YH. Apoptosis, proliferation and p53 gene expression of *H pylori* associated gastric epithelial lesions. *World J Gastroenterol* 2001; **7**: 779-782
2 **Michetti P**, Kreiss C, Kotloff KL, Porta N, Blano JL, Bachmann D, Herranz M, Saldinger PF, Corthesy-Theulaz I, Losonsky G, Nichols R, Simon J, Stolte M, Acherman S, Monath TP, Blum AL. Orla immunization with urease and *Escherichia coli* heat-labile enterotoxin is safe and immunogenic in *Helicobacter pylori*-infected adults. *Gastroenterology* 1999; **116**: 804-812
3 **Suganuma M**, Kurusu M, Okabe S, Sueoka N, Yoshida M, Wakatsuki Y, Fujiki H. *Helicobacter pylori* membrane protein 1: a new carcinogenic factor of *Helicobacter pylori. Cancer Res* 2001; **61**: 6356-6359
4 **Nakamura S**, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, Yao T, Tsuneyoshi M, Iida M, Fujishima M. Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of *Helicobacter pylori. Gut* 2001; **48**: 454-460
5 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 8298-8332
6 **Morgner A**, Miehlke S, Fischbach W, Schmitt W, Muller-Hermelink H, Greiner A, Thiede C, Schetelig J, Neubauer A, Stolte M, Ehninger G, Bayerdorffer E. Complete remission of primary high-grade B-cell gastric lymphoma after cure of *Helicobacter pylori* infection. *J Clin Oncol* 2001; **19**: 2041-2048
7 **Kate V**, Ananthakrishnan N, Badrinath S. Effect of *Helicobacter pylori* eradication on the ulcer recurrence rate after simple closure of perforated duodenal ulcer: retrospective and prospective randomized controlled studies. *Br J Surg* 2001; **88**: 1054-1058
8 **Muotiala A**, Helander IM, Pyhala L, Kosunen TU, Moran AP. Low biological activity of *Helicobacter pylori* lipopolysaccharide. *Infect Immun* 1992; **60**: 1714-1716
9 **Ogawa T,** Suda Y, Kashihara W, Hayashi T, Shimoyama T, Kusumoto S, Tamura T. Immunobiological activities of chemically defined lipid A from *Helicobacter pylori* LPS in comparison with Porphyromonas gingivalis lipid A and *Escherichia coli*-type synthetic lipid A (compound 506). *Vaccine* 1997; **15**: 1598-1605
10 **Matsuyama N,** Kirikae T, Kirikae F, Hashimoto M, Amanot K, Hayashi S, Hirai Y, Kubota T, Nakano M. Non-standard biological activities of lipopolysaccharide from *Helicobacter pylori. J Med Microbiol* 2001; **50**: 865-869
11 **Suda Y,** Kim YM, Ogawa T, Yasui N, Hasegawa Y, Kashihara W, Shimoyama T, Aoyama K, Nagata K, Tamura T, Kusumoto S. Chemical structure and biological activity of a lipid A compo-

nent from *Helicobacter pylori* strain 206. *J Endotoxin Res* 2001; **7**: 95-104

12 **Ogawa T,** Asai Y, Sakai Y, Oikawa M, Fukase K, Suda Y, Kusumoto S, Tamura T. Endotoxic and immunobiological activities of a chemically synthesized lipid A of *Helicobacter pylori* strain 206-1. *FEMS Immunol Med Microbiol* 2003; **36**: 1-7

13 **Venter P,** Lues JF. Extraction methods for lipopolysaccharides from *Escherichia coli* ATCC 25922 for quantitative analysis by capillary electrophoresis. *Int J Food Microbiol* 2003; **84**: 245-250

14 **Zherebylo OIe,** Moroz SM, Hvozdiak RI. Characteristics of bacterial lipopolysaccharides depending on extraction method. *Ukr Biokhim Zh* 2000; **72**: 51-55

15 **Mattsby-Baltzer I,** Mielniczuk Z, Larsson L, Lindgren K, Goodwin S. Lipid A in *Helicobacter pylori. Infect Immun* 1992; **60**: 4383-4387

16 **Skurnik M.** Molecular genetics, biochemistry and biological role of Yersinia lipopolysaccharide. *Adv Exp Med Biol* 2003; **529**: 187-197

17 **Luchi M,** Morrison DC. Comparable endotoxic properties of lipopolysaccharides are manifest in diverse clinical isolates of gram-negative bacteria. *Infect Immun* 2000; **68**: 1899-1904

18 **Salgado F,** Garcia A, Onate A, Gonzalez C, Kawaguchi F. Increased *in-vitro* and *in-vivo* biological activity of lipopolysaccharide extracted from clinical low virulence vacA genotype *Helicobacter pylori* strains. *J Med Microbiol* 2002; **51**: 771-776

19 **Pece S,** Giuliani G, Di Leo A, Fumarola D, Antonaci S, Jirillo E. Role of lipopolysaccharide and related cytokines in *Helicobacter pylori* infection. *Recenti Prog Med* 1997; **88**: 237-241

20 **Sakagami T,** Vella J, Dixon MF, O' Rourke J, Radcliff F, Sutton P, Shimoyama T, Beagley K, Lee A. The endotoxin of *Helicobacter pylori* is a modulator of host-dependent gastritis. *Infect Immun* 1997; **65**: 3310-3316

21 **Slomiany BL,** Slomiany A. Suppression of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide by peroxisome proliferator-activated receptor gamma activation. *IUBMB Life* 2002; **53**: 303-308

22 **Valente JF,** Alexander JW, Li BG, Noel JG, Custer DA, Ogle JD, Ogle CK. Effect of *in vivo* infusion of granulocyte colony-stimulating factor on immune function. *Shock* 2002; **17**: 23-29

23 **Tsitsilonis OE,** Tsavaris NB, Kosmas C, Gouveris P, Papalambros E. Immune changes in patients with colorectal cancer treated by adjuvant therapy with monoclonal antibody 17-1A: a pilot study. *J Chemother* 2003; **15**: 387-393

24 **Yamamoto T,** Kimura T, Ueta E, Tatemoto Y, Osaki T. Characteristic cytokine generation patterns in cancer cells and infiltrating lymphocytes in oral squamous cell carcinomas and the influence of chemoradiation combined with immunotherapy on these patterns. *Oncology* 2003; **64**: 407-415

25 **Zhao D,** Pothoulakis C. Rho GTPases as therapeutic targets for the treatment of inflammatory diseases. *Expert Opin Ther Targets* 2003; **7**: 583-592

26 **Meeuwsen S,** Persoon-Deen C, Bsibsi M, Ravid R, Noort JM. Cytokine, chemokine and growth factor gene profiling of cultured human astrocytes after exposure to proinflammatory stimuli. *Glia* 2003; **43**: 243-253

27 **Xing L,** Remick DG. Relative cytokine and cytokine inhibitor production by mononuclear cells and neutrophils. *Shock* 2003; **20**: 10-16

Edited by Zhu LH and Chen WW **Proofread by** Xu FM