Expression of HspA-UreB Fusion Protein of *Helicobacter pylori* and Its Immunocompetence

Liping Dai[1], Guangcai Duan[1], Qingtang Fan[2], Yuanlin Xi[1], Rongguang Zhang[1]

1. *Department of Epidemiology, College of Public Health, Zhengzhou University, Zhengzhou, Henan 450052, China*

2. *Henan Key Laboratory of Molecular Medicine, Zhengzhou, Henan 450052, China*

Abstract: Objective. To construct recombinant plasmid expressing HspA-UreB fusion protein of *H pylori*, and to determine its immunocompetence. **Methods.** The *hspA* and *ureB* genes were amplified by PCR from *H pylori* strain MEL-HP27 isolated in Zhengzhou and cloned directly into vector pET30a, and the recombinant plasmid was then transformed into *E. coli* BL21DE3. The recombinant plasmid was induced to express fusion protein HspA-UreB in *E. coli* by isopropylthio-β-D-galactoside (IPTG). The protein was analyzed by SDS-PAGE, purified by Ni^{2+} affinity chromatography, and immunized the mice. The immunoreactivity of the fusion protein were analyzed by Western blot. **Results.** In comparison with the reported corresponding sequence from Genbank, the nucleotide sequence homologies of the cloned *hspA* and *ureB* genes were 95.20 – 97.48% and 96.08 – 98.30%, and their putative amino acid sequence homologies were 95.76 – 97.46% and 98.77 – 99.82% for the two genes, respectively. The results of SDS-PAGE and optical density scanning indicated that the fusion protein was expressed by *pET30a-hspA-ureB*-BL21DE3 as a protein with *Mr* 82,100 of molecular weight and was 21% of the total bacterial proteins. The purity of fusion protein was 91%, and could be recognized by the serum from *H pylori* infected patients and mice immunized with purified HspA-UreB fusion protein. **Conclusion.** A recombinant plasmid expressing fusion protein HspA-UreB of *H pylori* was constructed and identified with good immunocompetence, and it suggested that HspA-UreB might be a potential vaccine antigen for controlling and treating *H pylori* infection. [Life Science Journal. 2006;3(2):21 – 26] (ISSN: 1097 – 8135).

Keywords: *Helicobacter pylori*; HspA-UreB; immunocompetence

Abbreviations: HapA: *Helicobacter pylori* adhesion A; HspA: heat shock protein subunit A; Lpp20: lipoprotein 20; NAP: neutrophil-activating protein; Omp: outer membrane protein; UreB: urease subunit B; VacA: vacoulating cytotoxin A

1 Introduction

Helicobacter pylori (*H pylori*) infection is a major cause of chronic active gastritis and most peptic ulcer diseases[1-3], and also closely related to gastric cancers[4,5]. This microorganism has been categorized as class I carcinogen by the World Health Organization[6]. For this reason, successful eradication of *H pylori* may be an important goal for this study. Currently, the treatment for *H pylori* infection involves antibiotic therapy, but this has some disadvantages such as increasing the expense and strains resistance[7,8]. An alternative approach is to develop a vaccine, which could eradicate *H pylori* infection[9,10]. Selection of antigenic epitope is critical in developing *H pylori* vaccine. The majority of studies attempting to produce a vaccine have focused on urease enzyme[11,12], heat shock protein[13,14] and vacuolating cytotoxin[15]. The protection afforded by a single antigen is not enough[16], but the two kinds antigen HspA and UreB combined could provide 100% protection for mice from being infected with *H pylori*.

In this study, the recombinant expression system for HspA-UreB fusion protein of *H pylori* was constructed. Immunoreactivity and immunogenicity of HspA-UreB fusion protein were further examined. The results of this study may contribute to the development of *H pylori* vaccines.

2 Materials and Methods

2.1 Materials

A clinical strain of *H pylori*, MEL-HP27 was isolated from a patient with chronic gastritis. Bacterial strain BL21 (DE3) and plasmid pET-30a were purchased from Novagen (Madison WI, USA). Primers for PCR amplification and restriction endonucleases *Sal* I, *Xho* I, *EcoR* I and T4

DNA ligase were purchased from Sangon (Shanghai, China). The Pyrobest™ high fidelity DNA polymerase and isopropyl-β-D-thiogalatopyranoside (IPTG) were purchased from Takara Company (Dalian, Jilin, China). Goat anti-mouse and goat anti-human IgG-HRP were purchased from Bangding Bioengineering company (Beijing, China). The mice were provided by the Center of Experimental Animal of Henan (Zhengzhou, Henan, China).

2.2 Construction of expression system *pET30a-hspA-ureB*-BL21 (DE3)

Genomic DNA of MEL-HP27 was extracted by conventional phenol-chloroform method. Oligonucleotide primers were designed based on the corresponding genomic sequence of international standard strain NCTC11637. The sequence of *hspA* sense primer with an endonuclease site of EcoR I was 5′-CCC GAA TTC ATG AAG TTT CAA CCA TTA-3′. The sequence of *hspA* antisense primer with an endonuclease site of *Sal* I was 5′-CGC GTC GAC GTG TTT TTT GTG ATC ATG AC- 3′. The sequence of *ureB* sense primer with an endonuclease site of *Sal* I was 5′- CC GTC GAC AAA AAG ATT AGC AGA AAA G-3′. The sequence of *ureB* antisense primer with an endonuclease site of *Xho* I was 5′- CGC CTC GAG CTA GAA AAT GCT AAA GAG-3′. PCR was performed with the hot start method.

The parameters for PCR were at 95 ℃ for 5 min, ×1; at 94 ℃ for 1 min, at 55 ℃ for *hspA* gene for 1 min (for 3 min for ureB gene), at 72 ℃ for 1 min, ×30; then at 72 ℃ for 10 min, ×1. The results of PCR were observed under UV light after electrophoresis. The expected sizes of target amplification fragments were 354 bp for *hspA* gene and 2710 bp for *ureB* gene.

PCR products of *hspA* gene digested with *EcoR* I and *Sal* I, and *ureB* gene digested with *Sal* I and *Xho* I, then were inserted into *EcoR* I and *Xho* I restriction fragments of the expression vector *pET30a* using T4 DNA ligase. The recombinant expression plasmids *pET30a-hspA-ureB* were transformed into competent *E. coli* BL21 (DE3), and the expression systems were named as *pET30a-hspA-ureB*-BL21 (DE3). The target fragments of *hspA* and *ureB* genes inserted in *pET30a* plasmid were sequenced by Sangon Company (China).

2.3 Expression, purification and identification of fusion proteins

The recombinant strains were incubated overnight at 37℃ while shaking in 5 ml LB medium with 100 μg/mL kanamycin, and the cell grew until the optical density at 600 nm reached 0.4 – 0.6. IPTG was added to a final concentration of 0.3 mmol/L. The cells growing for 4 h after induction were harvested by centrifugation at 4,000 g for 20 min. The molecular weight and output of HspA-UreB fusion protein were examined by SDS-PAGE. The serum of patient infected with *H pylori* and commercial goat-human HRP-IgG were used as the first and second antibodies to identify the immnuoreactivity of HspA-UreB. The recombinant *E. coli* cells growing in 50 ml LB medium with kanamycine for 3 h induced by IPTG harvested by centrifugation at 4,000 g for 20 min. The bacterial pellet resuspended in pure water was ultrasonically broken (300v, 4s×20), centrifugated at 12,000 g for 15 min. The recombinant HspA-UreB fusion protein was collected by Ni-NTA affinity chromatography and analyzed by electrophoresis in a 10% polyacrylamide gel.

2.4 Immunization of mice

Six to eight weeks old mice were immunized five times by hypodermic injection in the back at weekly intervals. Each dose consisted of 5 μg adjuvant. One week after the last immunization blood samples were taken from eyes. The sera were separated, and stored at −20 ℃ until assay.

2.5 Serum antibody response

The recombinant HspA-UreB fusion protein was electrophoresed as in SDS-PAGE and then were transformed to PVDF membrane. The mice sera immunized with HspA-UreB and goat anti-mouse HRD-IgG were used as the first and second antibodies to perform Western blot.

3 Results

3.1 PCR amplification of *H pylori hspA* and *ureB* genes

Target fragments of *hspA* and *ureB* genes with expected sizes amplified from DNA template of *H pylori* MEL-HP27 were shown in Figure 1.

3.2 Identification of recombinant plasmid

After extracting plasmids DNA from recombinant *E. coli* strains, the recombinant plasmids were digested by *EcoR* I or *Xho* I, and by *EcoR* I and *Xho* I simultaneously, then the digestive products were visualized on 10 g/L agarose gel eletrophorese (Figure 2). It demonstrated that recombinant plasmid contained the objective fusion gene.

3.3 Nucleotide sequence analysis

The nucleotide sequences of *hspA* and *ureB* gene in *pET30a-hspA-ureB* were listed in Figure 3 and Figure 4. The homologies of nucleotide and putative amino acid sequences of the cloned *hspA* gene compared with the published *hspA* sequences were

from 95.20% to 97.48% and from 95.20% to 97.46%, respectively (Table 1). The homologies of nucleotide and putative amino acid sequences of the cloned *uerB* gene were from 96.08% to 98.30% and from 98.77% to 99.65 % (Table 2).

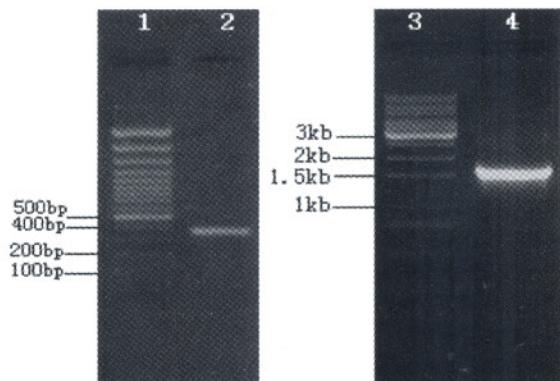

Figure 1. Target fragments of *hspA* and *ureB* genes amplified from *H pylori* strain MEL-HP27
Lane 1: 100 bp DNA ladder marker; Lane 2: PCR products of *hspA* gene; Lane 3: 1 kb DNA ladder marker; Lane 4: PCR products of *ureB* gene.

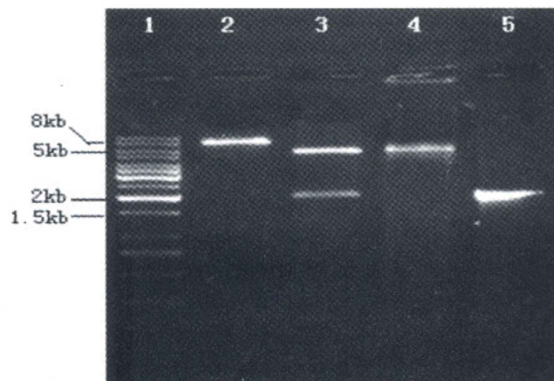

Figure 2. Identification of recombinant plasmid by restriction enzyme digestion
Lane 1: 1kb DNA ladder marker; Lane 2: recombinant plasmid digested by *EcoR* I; Lane 3: recombinant plasmid digested by *EcoR* I and *Xho* I; Lane 4: *pET30a* digested by *EcoR* I; Lane 5: products of *hspA-ureB* fusion gene from recombinant plasmid.

Table 1. Homology comparison of *H pylori hspA* gene sequences

H pylori strains compared with MEL-HP27	Different base pair	Homology of nucleotide	Different amoni acid	Homology of amoni acid
NCTC11637	9	97.48%	3	97.46%
26695	11	96.89%	3	97.46%
CH-CTX1	17	95.20%	5	95.76%
J99	10	97.18%	4	96.61%

Table 2. Homology comparison of *H pylori ureB* gene sequences

H pylori strains compared with MEL-HP27	Different base pair	Homology of nucleotide	Different amoni acid	Homology of amoni acid
NCTC11637	40	97.67%	3	99.47%
HPK5	29	98.30%	1	98.82%
26695	46	97.31%	6	98.95%
HP031	49	97.13%	5	99.12%
J99	67	96.08%	2	99.65%
CH-CTX1	51	97.01%	7	98.77%

3.4 Expression, purification and identification of HspA-UreB fusion protein

IPTG at concentration of 0.3 mmol/L efficiently induced the expression of HspA-UreB in the *pET30a-hspA-ureB*-BL21DE3 system. SDS-PAGE analysis showed that the clearly identifiable band with *Mr* 82,100 highly expressed fusion proteins, which was similar to that predicted.

The output of HspA-UreB fusion protein was approximate 21% of the total bacterial proteins (Figure 5). Among them, soluble substance accounted for 30% of supernatant. HspA-UreB fusion protein was further purified with Ni-NTA column, its final purity was 91%. Western blot also showed an identifiable blot band with *Mr* 82,100.

3.5 Antigenicity of recombinant HspA-UreB fusion protein

An immunized reacting band with the weight of *Mr* 82,100 appeared in the Western blot (Figure 6) in which the purified HspA-UreB fusion protein reacted against the serum of mouse immunized by HspA-UreB.

1 atgaagtttc taccattagg agaaagggtc ttagtagaaa gacttgaaga agagaacaaa
61 accagttcag gcatcatcat ccctgataac gctaaagaaa agcctttaat gggcgtagtc
121 aaagcggtta gccataaaat cagcgagggt tgcaaatgcg ttaaagaagg cgatgtgatc
181 gcttttggca aatacaaagg cgcagaaatc gttttagacg gcgttgaata catggtgcta
241 gagctagaag acattctagg tattgtgggc tcaggctctt gttgtcatac aaatagtcat
301 gaccataaac atgctaaaga gcatgaagct tgctgtcatg atcacaaaaa acactaa

Figure 3. *hspA* nucleotide sequence of *H pylori* strain MEL-HP27

1 atgaaaaaga ttagcagaaa agaatatgtt tctatgtatg gccctactac aggcgataaa
61 gtgagattgg gcgatacaga cttgatcgct gaagtagaac atgactacac catttatggc
121 gaagagctta aattcggtgg cggtaaaact ttgagagaag gcatgagcca atccaacaac
181 cctagcaaag aagaactgga tttaatcatc actaacgctt taatcgtgga ttacaccggt
241 atttataaag cggatattgg tattaaagat ggcaaaatcg ctggcattgg caaaggcggc
301 aacaaagaca tgcaagatgg cgttaaaaac aatcttagcg tgggtcctgc tactgaagcc
361 ttagctggtg aaggtttgat cgtaactgct ggtggtattg acacacacat ccacttcatc
421 tccccccaac aaatccctac agcttttgca agcggtgtaa caacgatgat tggtggcgga
481 actggccctg ctgatggcac taacgcaacc actatcactc caggcagaag aaatttaaaa
541 tggatgctca gagcggctga agaatattct atgaatttag gtttcttagc taaaggtaac
601 gcttctaatg atgcgagctt agccgatcaa attgaagccg gtgcgattgg ctttaaaatc
661 catgaagact ggggaacaac tccttctgca atcaatcatg cgttagatgt tgcggacaaa
721 tacgatgtgc aagtcgctat ccatacggac actttgaatg aagccggttg tgtagaagac
781 actatggcag ccattgccgg acgcactatg cacactttcc acactgaagg cgctggtggc
841 ggacacgctc ctgatatcat taaagtagcc ggcgaacaca acattctgcc cgcttccact
901 aaccccacta tccctttcac tgtgaataca gaagcagaac acatggacat gcttatggtg
961 tgccaccact tggataaaag cattaaagaa gatgttcagt tcgctgattc aaggatccgc
1021 cctcaaacca ttgcggctga agacactttg catgacatgg ggattttctc aatcactagt
1081 tctgactctc aagctatggg tcgtgtgggt gaagttatca ccagaacttg gcaaacagct
1141 gacaaaaaca aaaaagaatt tggccgcttg aaagaagaaa aaggcgataa cgacaacttc
1201 agaatcaaac gctacttgtc taaatacacc attaacccag cgatcgctca tgggattagc
1261 gagtatgtag gttctgtaga agtgggcaaa gtggctgact tggtattgtg gagtccagca
1321 ttctttggcg tgaaacccaa catgatcatc aaaggtgggt ttattgcatt gagtcaaatg
1381 ggcgatgcga acgcttctat ccctacccca caaccagttt attacagaga aatgttcgct
1441 catcatggta aagccaaata cgatgcaaac atcacttttg tgtctaaagc ggcttatgac
1501 aaaggcatta aagaagaatt agggcttgaa agacaagtgt tgccggtaaa aaattgcaga
1561 aacatcacta aaaaagacat gcaattcaac gacactaccg ctcacattga agtcaatcct
1621 gaaacttacc atgtgttcgt ggatggcaaa gaagtaactt ctaaaccagc cactaaagtg
1681 agcttggcgc aactctttag cattttctag

Figure 4. *ureB* nucleotide sequence of *H pylori* strain MEL-HP27

4 Discussion

Immune protection function of several *H pylori* antigens has been studied and investigated, such as UreB[17,18], HspA[19], VacA[15], Catalase[20,21]. Several studies demonstrated that three of *H pylori* antigens, such as Lpp20[22,23], NAP[24,25], HpaA[26], Omp[27] are also excellent and ideal antigens that can be potentially used for the development of *H pylori* vaccine. However, some researches indicated that immune protection function of combined antigens was better than single antigen. Recently, some researchers study poly-antigen genetic engineering vaccine of *H pylori* in China[27,28]. This research selected two kinds of antigens with effective immunization: HspA and UreB, to construct the expression system for *hspA-ureB* fusion gene, and to determine the immunogenicity and immunoreactivity of HspA-UreB fusion protein. UreB, used as a candidate antigen for *H pylori* genetic engineering vaccine, has advantages of high sequence conservation, high frequency of distribution, large expression in different isolates, strong antigenicity due to its big molecular mass, and granular structure and exposure on the

surface of bacteria[29,30]. HspA is another candidate antigen for *H pylori* vaccine, which is termed as "molecular chaperone" because they assist in post-translational assembly, secretion, and stability of oligomeric protein structures[31].

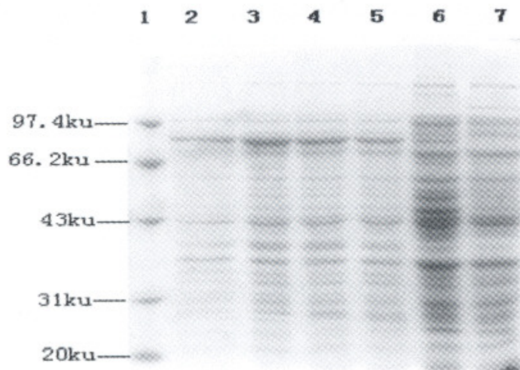

Figure 5. SDS-PAGE analysis of HspA-UreB induced by IPTG at different time
Lane 1: molecular weight marker; Lanes 2-5: BL21 (DE3) with *pET-hspA-ureB* induced for 4, 3, 2 and 1 h; Lane 6: BL21 (DE3) with *pET-hspA-ureB* uninduced for 4 h; Lane 7: BL21 (DE3) with *pET30a* induced for 4 h.

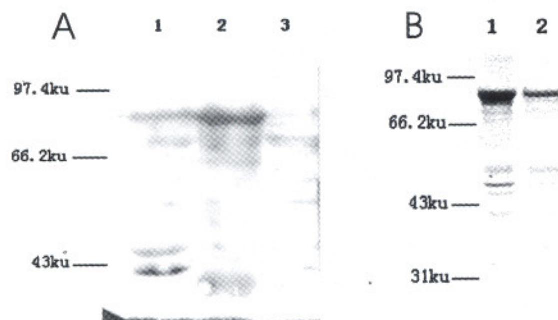

Figure 6. Western blot result of mouse serum against recombinant HspA-UreB
Lane A1: BL21 (DE3) with *pET-hspA-ureB* uninduced for 4 h;
Lane A2: BL21 (DE3) with *pET-hspA-ureB* induced for 4 h;
Lane A3: BL21 (DE3) with *pET30a* induced for 4 h;
Lane B1: unpurified HspA-UreB; Lane B2: purified HspA-UreB.

In this study, the recombinant plasmids expressing HspA-UreB fusion gene of *H pylori* were constructed. The *hspA* gene cloned from *H pylori* strain MEL-HP27 showed high homologies of the nucleotide and putative amino acid sequences compared with five published corresponding sequences (Table 1). Similarly, the homologies of nucleotide and putative amino acid sequences of the clone *ureB* gene from *H pylori* strain MEL-HP27 were quite high when compared with the published corresponding sequences (Table 2).

The results of SDS-PAGE demonstrated that the constructed expression system *pET30a-hspA-ureB*-BL21 (DE3) efficiently produced the target fusion protein. The most output of fusion protein HspA-UreB within whole cell protein was about 21% of the total bacterial proteins, which is beneficial to industrial production.

Western blot assay was performed in this study to confirm that the purified fusion protein HspA-UreB could be recognized by the serum from patient infected with *H pylori* and also be recognized by the sera from mice immunized with purified fusion protein. HspA-UreB exhibited favorable immunogenicity and immunoreactivity.

In conclusion, HspA-UreB is excellent and ideal candidate antigen that can be potentially used for the development of *H pylori* vaccine.

Correspondence to:
Guangcai Duan
Department of Epidemiology
College of Public Health
Zhengzhou University
Zhengzhou, Henan 450052, China.
Telephone: 86-371-6691-1354
Email: gcduan@public2.zz.ha.cn

References

1. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1: 1273-5.
2. Mendall MA. Transmission of *Helicobacter pylori*. Semin Gastrointest Dis 1997; 8(3): 113-23.
3. Blaser MJ. Gastric campylobacter-like organisms, gastritis, and peptic ulcer disease. Gastroenterol 1987; 93(2): 371-83.
4. Zhang QX, Lin SR. Research of *Helicobacter pylori* infection in precancerous gastric lesions. World J Gastroenterol 2000; 6: 428-9.
5. Gao H, Wang JY, Shen XZ, et al. Effect of *Helicobacter pylori* infection on gastric epithelial cell proliferation. World J Gastroenterol 2000; 6: 442-4.
6. Vainio H, Heseltine E, Wilbourn J. Priorities for future IARC monographs on the evaluation of carcinogenic risks to humans. Environ Health Perspect 1994; 102(6-7): 590-1.
7. Harris A. Treatment of *Helicobacter pylori*. World J Gastroenterol 2001; 7: 303-7.
8. Hua JS, Bow H, Zheng PY, et al. Prevalence of primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in Singapore. World J Gastroenterol 2000; 6: 119-21.
9. Bai Y, Wang JD, Zhang YL. Construction of the attenuated Salmonella typhimurium strain expressing *Helicobacter pylori* conservative region of adhesin antigen. Chin J Biotech 2003; 19(4): 433-8.
10. Mastroeni P, Bowe F, Cahill R, et al. Vaccines against

gut pathogens. Gut 1999;5: 633–5.

11. Lee MH, Roussel Y, Wilks M, et al. Expression of *Helicobacter pylori* urease subunit B gene in Lactococcus lactis MG1363 and its use as a vaccine delivery system against *H. pylori* infection in mice. Vaccine 2001; 19 (28–29):3927–35.
12. Mao YF, Yan J. Construction of prokaryotic expression system of ureB gene from a clinical *Helicobacter pylori* strain and identification of the recombinant protein immunity. World J Gastroenterol 2004; 10(7): 977–84.
13. Todoroki I, Joh T, Watanabe K, et al. Suppressive effects of DNA vaccines encoding heat shock protein on *Helicobacter pylori*-induced gastritis in mice. Biochem Biophys Res Commun 2000; 277(1): 159–63.
14. Kansau I, Guillain F, Thiberge JM, et al. Nickel binding and immunological properties of the C-terminal domain of the *Helicobacter pylori* GroES homologue (HspA). Mol Microbiol 1996; 22(5): 1013–23.
15. Rossi G, Ruggiero P, Peppoloni S, et al. Therapeutic vaccination against *Helicobacter pylori* in the beagle dog experimental model: safety, immunogenicity, and efficacy. Infect Immun 2004; 72(6): 3252–9.
16. Ferrero RL, Thibrge JM, Kansau I, et al. The GroES homolog of *Helicobacter pylori* confers protective immunity against mucosal infection in mice. Proc Natl Acad Sci USA 1995; 92(14): 6499–503.
17. Fujii R, Morihara F, Fukushima K, et al. Recombinant antigen from *Helicobacter pylori* urease as vaccine against *H pylori*-associated disease. Biotechnol Bioeng 2004; 86 (7): 737–46.
18. Metzger WG, Mansouri E, Kronawitter M, et al. Impact of vector-priming on the immunogenecity of a live recombinant Salmonella enterica serovar typhi Ty21a vaccine expressing urease A and B from *Helicobacter pylori* in human volunteers. Vaccine 2004; 22(17–18): 2273–7.
19. Jiang Z, Huang AL, Tao XH, et al. Construction and characterization of bivalent vaccine candidate expressing HspA and M(r)18,000 OMP from *Helicobacter pylori*. World J Gastroenterol 2003; 9(8): 1756–61.
20. Chen M, Chen J, Liao W, et al. Immunization with attenuated Salmonella typhimurium producing catalase in protection against gastric *Helicobacter pylori* infection in mice. Helicobacter 2003; 8(6):613– 25.
21. Miyashita M, Joh T, Watanabe K, et al. Immune responses in mice to intranasal and intracutaneous administration of a DNA vaccine encoding *Helicobacter pylori*-catalase. Vaccine 2002; 20(17–18): 2336–42.
22. Keenan J, Neal S, Allardyce R, et al. Serum-derived IgG1-mediated immune exclusion as a mechanism of protection against *H pylori* infection. Vaccine 2002;20(23–24):2981–8.
23. Keenan J, Oliaro J, Domigan N, et al. Immune response to an 18-kilodalton outer membrane antigen identifies lipoprotein 20 as a *Helicobacter pylori* vaccine candidate. Infect Immun 2000; 68(6): 3337– 43.
24. Dundon WG, Nishioka H, Polenghi A, et al. The neutrophil-activating protein of *Helicobacter pylori*. Int J Med Microbiol 2002; 291(6–7):545– 50.
25. Satin B, Del Giudice G, Della Bianca V, et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med 2000;191(9):1467–76.
26. Lundstrom AM, Bolin I, Bystrom M, et al. Recombinant HpaA purified from *Escherichia coli* has biological properties similar to those of native *Helicobacter pylori* HpaA. APMIS 2003; 111(3):389–97.
27. Jiang Z, Huang AL, Pu D, et al. Construction, expression and antigenicity of bivalent vaccine candidate of human *Helicobacter pylori*. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2004; 20(1): 62– 6.
28. Jiang Z, Pu D, Huang AL, et al. Construction, expression and antigenic study of bivalent vaccine candidate with 26,000 OMP and heat shock protein A of human *Helicobacter pylori*. Zhonghua Yi Xue Za Zhi 2003; 83 (10): 862–7.
29. Dieterich C, Bouzourene H, Blun AL, et al. Urease-based mucosal immunization against *Helicobacter pylori* infection induced corpus atrophy in mice. Infect Immune 1999; 67: 6206–9.
30. Ernst JD. Toward the development of antibacterial vaccines: report of a symposium and workshop. Organizing Committee Clin Infect Dis 1999; 29: 1295–302.
31. Suerbaum S, Thiberge JM, Kansau I, et al. *Helicobacter pylori* hspA-hspB heat-shock gene cluster: nucleotide sequence, expression, putative function and immunogenicity. Molecular Microbio 1994; 14(5): 959–74.

Received March 10, 2006