Expression of Helicobacter pylori AlpA protein and its immunogenicity

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CONCLUSION: Adhesin AlpA recombinant protein may be a potential vaccine for control and treatment of H pylori infection.

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Key words: H pylori, Immunogenicity

INTRODUCTION
Helicobacter pylori (H pylori) infection is the major cause of chronic active gastritis and peptic ulcer disease[3], and is also closely related with gastric cancers such as adenocarcinomas, mucosa-associated lymphoid tissue lymphoma and primary gastric non-Hodgkin’s lymphoma[5]. This organism has been categorized as a class I carcinogen by the World Health Organization, and direct evidence of its carcinogenicity was recently demonstrated in China. In addition, seroprevalence studies indicate that H pylori infection is also associated with cardiovascular, respiratory, extra-gastroduodenal digestive, autoimmune diseases. Successful eradication of H pylori is thus an important goal. Currently, treatment involves antibiotic therapy, but this has associated problems such as low patient compliance and an increase of resistant strains. An alternative approach is to develop a vaccine, which would not only clear the organism, but also protect against reinfection.

In the development of H pylori vaccine, the candidate vaccine antigens adopted currently such as urease, vacuolating cytotoxin and catalase[6] focused basically on blocking toxicity factors of H pylori, while few candidate vaccine antigens focused on adhesins, which are closely associated with H pylori colonizing in human gastric mucosa by adhering to mucous epithelial cells and the mucus layer lining the gastric epithelium. It is probably a useful attempt to discover protective antigens based on adhesin. Currently, four adhesins have been recognized[7-9], including H pylori adhesin BabA[10] whose receptor has been determined till date and other three adhesins of AlpA[11], AlpB[12] and HopZ[13]. No concerned study has been reported about AlpA in China. So in this study, the recombinant plasmid of H pylori AlpA gene was constructed for development of H pylori vaccine.

MATERIALS AND METHODS

Materials
Bacterial strain BL21(DE3) and plasmid pET-22b(+) were provided by the Institute of Biotechnology, Academy of

Abstract
AIM: To construct a recombinant strain which expresses adhesin AlpA of Helicobacter pylori (H pylori) and to study the immunogenicity of adhesin AlpA.

METHODS: Gene Ab, which was amplified from H pylori chromosomal DNA by PCR technique, was sequenced and the biological information was analyzed, and inserted into the Nco I and Not I restriction fragments of the expression vector pET-22b(+) using T4 DNA ligase. The resulting plasmid pET-AlpA was transformed into competent E.coli BL21(DE3) cells using ampicillin resistance for selection. Recombinant strains were incubated in 5 mL LB with 100 μg/mL ampicillin overnight at 37 °C. Sonication of BL21(DE3)pET-22b(+)/AlpA was analyzed by Western blot to detect AlpA immunogenicity.

RESULTS: The gene encoding AlpA protein was amplified by PCR with chromosomal DNA of H pylori Sydney strain (SS1) as templates. It revealed that AlpA DNA fragment amplified by PCR had approximately 1 500 nucleotides, compatible with the previous reports. The recombinant plasmid pET-22b(+)/AB was successfully constructed. DNA sequencing showed one open reading frame with the length of 588 bp. It encoded seven conservative regions that showed good antigenicity and hydrophobicity by Parker and Wellung method. Furthermore, INTERNET EXPASY, NN_PREDICT and ISREC predicted that it was a porin-like structure consisting of β-pleated sheets that were embedded in the outer membrane. BLAST analyzed 836 767 protein sequences and found that the similar sequences were all belonging to H pylori OMP sequences. SDS-PAGE and scan analysis showed that the molecular weight of AB was 22.5 kDa and recombinant protein amounted to 29% of the total bacterial protein, among which dissolved expression amounted to 21.9% of sonicated supernatant. The RAB purity amounted to 96% through affinity chromatography. Western blot analysis of RAB confirmed that it could be specially recognized by serum form rabbit immunized with AlpA and H pylori infected.

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MATERIALS AND METHODS

Materials
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Military Medical Sciences. *H pylori* SS1 was preserved in this research institute. Restriction enzymes *Nco* I, *Nde* I and T4 DNA ligase, Vent DNA polymerase and isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from New England Biolabs. Goat anti-rabbit and goat anti-human IgG-HRP were purchased from Huamei Bioengineering Company, China, and His-Tag precolum from Invitrogen. The serum from rabbits immunized with AlpA was donated by Odenbreit et al.[13]. Serum samples were obtained from *H pylori* positive and negative patients who underwent urease test, pathologic examination and germiculture at the Endoscope Center of this institute. Other reagents were analytically pure reagents produced in China.

**Recombinant DNA techniques**

All restriction enzyme digestions, ligations and other common DNA manipulations, unless otherwise stated, were performed by standard procedures[1]. The genome of *H pylori* was prepared from cells collected from the colonies on the agar plate. The gene of *H pylori* by PCR (Techne PROGENE) using primers AlpA1 (5'-TG GCC ATG GAT TGC GCT AGC-3') as downstream primer and AlpA2 (5'-AG TGC GGC CGC GAA TGA ATA CCC ATA AGA -3') as upstream primer and AlpA2 (5'- AG TGC GGC CGC GAA TGA ATA CCC ATA AGA -3') as upstream primer and AlpA2 (5'- AG TGC GGC CGC GAA TGA ATA CCC ATA AGA -3') as downstream primer as described in the literature[11]. AlpA1 and AlpA2 contained *Neo* I and *Not* I sites, respectively. PCR was performed with the hot start method. The PCR condition was initial denaturing at 95℃ for 30 s, each cycle of amplification consisted of denaturing at 95℃ for 30 s, annealing at 55℃ for 30 s and polymerization at 72℃ for 50 s and further polymerization for 10 min after 35 PCR cycles. The PCR products were harvested from agarose gel, digested with *Neo* I and *Not* I, and inserted into the *Neo* I and *Not* I restriction fragments of the expression vector pET-22b(+) using T4 DNA ligase. The resulting plasmid pET-AlpA was amplified into competent *E.coli* BL21 (DE3) cells using ampicillin resistance for selection. The alkaline lysis method was used for large-scale preparations of the recombinant plasmid and the plasmid was identified by restriction enzymes. DNA sequence was performed with the DNA automatic sequencer.

**Induction of expression and SDS-polyacrylamide gel electrophoresis**

Recombinant strains were incubated in 5 mL LB with 100 μg/mL ampicillin overnight at 37℃. Fifty microliters LB was inoculated and the cells grew until the optical density at 600 nm reached 0.4-0.6. IPTG was added to the cultures at the final concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mmol/L, respectively. *E.coli* cells were harvested after 3 or 5 h by centrifugation at 12 000 g for 10 min. The pellet was resuspended in 1 mL 30 mmol/L Tris buffer (pH 8.0) containing 1 mmol/L EDTA (pH 8.0) and 20% sucrose. The suspension was put on ice for 10 min, then centrifuged for 10 min at 12 000 g, and the resulting supernatant contained proteins from the periplasm. The resulting pellet was resuspended in 5 mL 50 mmol/L Tris buffer (pH 8.0) containing 2 mmol/L EDTA, 0.1 mg/mL lysozyme and 1% Triton X-100. The suspension was incubated at 30℃ for 20 min and then sonicated on ice until it became clarified. The lysate was centrifuged at 12 000 g for 15 min at 4℃. Whole-cell lysates, sonicated supernatant, inclusion body, osmotic shock liquid of recombinant strains expressing *H pylori* AlpA were analyzed by electrophoresis in a 10% polyacrylamide gel.

**Western blot analysis on sonication of BL21 (DE3) pET-22b(+) /AlpA**

Sonication of BL21(DE3)pET-22b(+)/AlpA was analyzed by Western blot to detect AlpA immunogenicity. Protein was separated on 10% SDS-PAGE gel using a mini-gel apparatus (Bio-Rad) and transferred to nitrocellulose membranes using a semi-dry blot system (Biotech Fischer). The polyclonal rabbit antiserum and sera from patients infected with *H pylori* were pre-absorbed with BL21(DE3)pET-22b(+) sonication, to remove non-specific antibodies diluted at 1:50. The membrane was incubated for 90 min at 37℃, washed thrice for 15 min with TBS/Tween-20 (TTBS), and then incubated for 1 h with goat-anti rabbit IgG alkaline phosphates conjugate at room temperature. After washing thrice with TTBS, BCIP/NBT was used to visualize bound antibodies.

**RESULTS**

**PCR amplification of *H pylori* AlpA gene**

The gene encoding AlpA protein was amplified by PCR with chromosomal DNA of *H pylori* Sydney strain (SS1) as templates. The cloned products were electrophoresed and visualized on 8 g/L agarose gel (Figure 1). It revealed that AlpA DNA fragment amplified by PCR had approximately 1 500 nucleotides, compatible with the previous reports[11].

**Construction of recombinant plasmid and restriction enzyme confirmation**

After the PCR products and pET-22b(+) plasmid were cut by *Not* I and *Not* I, directional cloning was performed, resulting in a recombinant plasmid named pET-22b(+)/AlpA. The recombinant plasmids pET-22b(+) /AlpA were all digested by *Not* I or *Not* I, and by *Not* I and *Neo* I simultaneously, and then the digestive products were visualized on 8 g/L agarose gel electrophoreses (Figure 2). It demonstrated that recombinant plasmid contained the objective gene.
Expression of AlpA gene in Escherichia coli

Whole-cell sonication, sonicated supernatant, osmotic shock liquid of recombinant strains expressing H pylori AlpA genes were analyzed by electrophoresis in a 10% polyacrylamide gel for detection of fusion proteins (Figure 3). The result showed that the clearly identifiable band was 56 500 ku. Afterwards, two close homologous genes were identified, designated as AlpA and AlpB, encoding outer membrane (OM) proteins of 518 amino acids each[11]. They are members of the outer membrane protein supergene family identified in the H pylori 26 695 complete genome sequence. Transposon insertion mutagenesis, immunoblotting and primer extension studies indicated that both genes were organized in an operon, but no obvious consensus promoter sequence was found upstream of the transcriptional start site. The C-terminal portion of both proteins was predicted to form a porin-like barrel in the outer membrane, consisting of 14 transmembrane amphipathic strands. Preincubation of H pylori with an antiserum raised against the AlpA fusion protein completely blocked binding of H pylori to the gastric tissue sections, but an antiserum raised against a H pylori urease B fusion protein did not[11]. These data suggested that AlpA might be necessary for specific adhesion of H pylori to human gastric tissues and AlpA must be located to the gastric surface exposed and well conserved, might be excellent vaccine candidates[15,16]. AlpA accords with the criteria mentioned above. Furthermore, AlpA as a candidate component of gene engineering vaccines possess the following virtues. AlpA

DISCUSSION

In 1996, Odenbreit isolated and characterized a chromosomal locus of H pylori previously identified by transposon shuttle mutagenesis as being involved in adhesion of the pathogen to gastric epithelial cells[4]. Afterwards, two close homologous genes were identified, designated as AlpA and AlpB, encoding outer membrane (OM) proteins of 518 amino acids each[11]. These proteins, which are H pylori family of 32 genes in H pylori surface exposed and well conserved, might be excellent vaccine candidates[15,16]. AlpA accords with the criteria mentioned above. Furthermore, AlpA as a candidate component of gene engineering vaccines possess the following virtues. AlpA

Sequence analysis of cloned AlpA nucleotide

The nucleotide sequence of cloned genes inserted in pET22b (+) was analyzed by automated sequencing across the cloning junction, using the universal primer T7. The cloned genes contained 1 512 nucleotides coding a putative protein consisted of 504 amino acid residues with a calculated molecular mass of AlpA. The homogenicity was 97.3% between them.

Expression of AlpA gene in Escherichia coli

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is a kind of protein so that a gene engineering clone expressing AlpA can be constructed to realize scale-up production and purification. Although AlpA as an adhesion is an important component attending Helicobacter pylori infection and AlpA itself is lack of toxicity and cross-reactivity with human tissue antigens. AlpA exists in H pylori isolated from multiple geographic locations. These virtues also fit the criteria for its selection and purification. Although AlpA as an adhesion is an essential for the adhesion of Helicobacter pylori and evaluation of the adherence activity of AlpA. Construction of the E.coli clone expressing adhesin BabA of Helicobacter pylori and evaluation of the adherence activity of BabA.  

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