

Research paper

Production of mouse monoclonal antibodies against *Helicobacter pylori* Lpp20 and mapping the antigenic epitope by phage display library

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Received 13 November 2006; received in revised form 19 March 2007; accepted 12 May 2007

Available online 14 June 2007

Abstract

Lpp20, an outer membrane protein of *Helicobacter pylori* (*H. pylori*), has been identified as an immunodominant antigen. To obtain mouse monoclonal antibodies (mAbs) against it and to map its antigenic epitope is potentially to develop a vaccine for prevention and treatment of *H. pylori* infection. In our study, the Lpp20 gene was obtained from *H. pylori* genomic DNA by PCR (GenBank accession no. DQ106902), cloned into pGEX-4T-1 vector and expressed in *Escherichia coli* (*E. coli*) as a recombinant fusion protein with glutathione-S-transferase (GST), which was purified by GST-affinity chromatography. mAbs were produced by the hybridoma technique using Lpp20-GST as the immunogen. Using mAb as the target molecule and immunoscreening phage-displayed random dodecapeptide library (Ph.D.-12), the positive phage clones were sequenced and analyzed. Phage clones were chosen to immunize mice to evaluate the potential of phagotopes as effective vaccines. One mimotope (SWPLYSDASGLG) showed a good match with the Lpp20 proteins at 114–117aa (DASG) and the serum of mice induced by the phage clone clearly recognized Lpp20 protein. Our work suggests that the antigenic epitope could be mapped through screening the phage-displayed peptide libraries with mAb and a mimotope of Lpp20 providing an alternative approach for the diagnosis and development of a vaccine for *H. pylori*.

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Keywords: *Helicobacter pylori*; Monoclonal antibody; Phage display library; Epitope

1. Introduction

Helicobacter pylori (*H. pylori*), a curved bacilli discovered in the stomach of patients with gastritis and

peptic ulceration (Marshall and Warren, 1984), is the main cause of various gastroduodenal diseases such as ulcer and cancer. Approximately half of the world's population is infected with *H. pylori* (Dunn et al., 1997). Antibiotics are still the most effective treatment for *H. pylori* infection, but antibiotic resistance is becoming a problem. Thus, it is of great interest to find alternative ways to control the infection. Vaccination has become one of the most promising approaches against *H. pylori*

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infection. However, no antibodies are currently available to immune exclude *H. pylori* from the stomachs of infected individuals.

Phage display technology is widely used to rapidly identify antibodies against molecular targets in vitro (Smith and Petrenko, 1997; Kouzmitcheva et al., 2001). For the past few years, the phage-displayed random peptide libraries have been successfully used to mimic epitopes of antigens (minotopes) and induce humoral immune response in animals (Bastien et al., 1997; Meola et al., 1995), especially mimicking the carbohydrate epitope of human-immunodeficiency-virus (Agadjanyan et al., 1997) and *Neisseria meningitidis* (Grothaus et al., 2000). Thus, phage-displayed mimotopes have been proposed as a potential new generation of peptide vaccines.

Lpp20, a conserved *H. pylori* lipoprotein, is an immunodominant antigen (Keenan et al., 2000, 2002; Bakos et al., 2003), which can be used for the design of a prophylactic vaccine for *H. pylori*. However, the epitope of Lpp20 has not yet been defined. In the present study, we applied phage display technology to map the epitope of *H. pylori* Lpp20 using specific mAb. The selected phage clones were immunoscreened from a phage-displayed dodecapeptide library and the inserting peptide sequences were sequenced and analysed. The mimotopes were used to immunize BALB/c mice and the immune responses for phageto were evaluated.

2. Materials and methods

2.1. Growth of bacterial cells

H. pylori NCTC11639 strain was obtained from Department of Microbiology, Southern Medical University (Guangzhou, China). This cell line was routinely cultured on Columbia agar plates supplemented with 7% saponin-lysed horse blood, 0.004% triphenyltetrazolium chloride, and Dent selective supplement (Oxoid, Basingstoke, UK) for 3 days at 37 °C under a microaerophilic atmosphere (5% O₂, 15% CO₂ and 80% N₂). Then a colony was picked up and inoculated into 20 ml of Brucella broth (Becton Dickinson, Cockeysville, MS) which contained 0.1% β -cyclodextrin supplemented with 5% (v/v) horse serum. After 24 h, 2 ml of culture was transferred to 40 ml of fresh medium and the same processes were repeated twice. Finally, 1 ml of the incubated medium containing the cells, which were mostly spiral rather than coccoid, was plated on Brucella agar (Becton Dickinson) containing 7% (v/v) defibrinated horse blood and cultured for an additional 3 days at 37 °C in a microaerophilic atmosphere. Bacterial cells were harvested and washed twice with cold phosphate-

buffered saline (PBS, 25 mM sodium phosphate, pH 7.2, 0.9% NaCl). Then the cells were sedimented by centrifugation (5000 $\times$ g for 10 min at 4 °C), and the cell pellet was stored at –80 °C.

2.2. Cloning and purification of Lpp20 protein

The genomic DNA of *H. pylori* was prepared using a Takara kit (Takara, Japan) according to the manufacturer's instructions. The extracted genomic DNA was then used as the template for amplification of the Lpp20 protein coding region using a Taq DNA polymerase PCR kit (Takara, Japan). Two primer sequences corresponding to the 5' and 3' ends of the coding gene plus *Eco*RI and *Sal*I restriction sites were:

P1: 5'GCCGAATTCATGAAAAATCAAGTTA-3'
P2: 5'GCCGTCGACCTACTTTTAAACCATGC-3'

The PCR product was cloned into the expression vector pGEX-4T-1 (Amersham Biosciences). The plasmid was then transformed into *Escherichia coli* strain TOP10 (Invitrogen BV, Leek, The Netherlands) and Lpp20 protein expression was induced with 1 mM isopropyl- β -D-1-thiogalactopyranoside when the cells were grown at log phase at room temperature. After 4 h, the cells were harvested by centrifugation and washed with ice-cold PBS containing 5 mM EDTA and 2 mM PMSF. All subsequent procedures were performed at 4 °C.

To purify the expressed Lpp20 protein, the cells were resuspended in PBS containing 5 mM EDTA and 2 mM PMSF and lysed by sonication on ice followed by centrifugation at 10,000 rpm for 20 min to sediment the cell debris. The supernatant was applied to glutathione Sepharose 4B (Pharmacia, Sweden) to purify Lpp20 protein according to the manufacturer's instructions. The Lpp20 samples were analyzed by electrophoresis on a 10% SDS-polyacrylamide gel. The gels were stained for protein with 0.25% Coomassie Blue or transferred to a nitrocellulose membrane (Millipore Co., Bedford, MA, USA) and probed with diluted human serum (1/50 dilution with PBS) from a patient diagnosed with *H. pylori* infection (positive serum) and a normal donor without *H. pylori* infection. Detection was performed with goat anti-human antibody linked to horseradish peroxidase (1/10,000) followed by staining with 3-amino-9-ethylcarbazole (Sigma, USA) as a chromogen.

2.3. Production of the monoclonal antibody

BALB/c female mice, 4–6 weeks old, were purchased from the Animal Center of Southern Medical University.

The mice were immunized by intraperitoneal injection with 50 µg of purified Lpp20-GST which was emulsified with an equal volume of Freund's complete adjuvant. Three further injections were administered using incomplete adjuvant every 2 weeks. Three days after the last injection, the spleen cells of the mice were harvested and fused with the Sp 2/0 mouse myeloma cell line. After 10–14 days, the culture supernatants were screened with an ELISA test in which the solid phase was coated with the recombinant Lpp20-GST (2 µg/ml) used for the immunization and GST protein expressed by pGEX-4T-1 vector (2 µg/ml). In the screening process, the mAbs reacting with coated Lpp20-GST but not with coated GST were selected. By twice limiting dilution, positive colonies were subcloned. Ascitic fluids were harvested from the mice primed with a 0.5 ml intraperitoneal injection of Pristane and then injected with 10^6 hybridoma cells. The class and subclass of each mAb were determined using a mouse monoclonal antibody isotyping kit (HyCult Biotechnology B.V., Netherlands). The mAbs were purified from the mouse ascitic fluids using a protein G-Sepharose 4FF (Pharmacia, Uppsala, Sweden) column according to the manufacturer's instructions to remove components which might interfere with the biopanning experiments.

The antibody titers were determined by ELISA methods. Briefly, purified Lpp20-GST antigen was coated onto the microtiter plates (100 µl/well, 5 µg/ml in coating solution, 0.1 M sodium bicarbonate, and pH 9.6) (Corning Inc, USA) and incubated overnight at 4 °C. Then the supernatants of the cell culture as well as the ascitic fluids were incubated with pre-coated Lpp20-GST (100 µl/well) for 2 h at 37 °C. Then goat anti-mouse IgG HRP (1/10,000 dilution with washing buffer) (Sigma, USA) was used to detect Lpp20-GST bound primary monoclonal antibodies and *O*-phenylenediamine dihydrochloride was used as substrate (Pierce Chemicals) for horseradish peroxidase; the reaction was monitored at the absorption of 492 nm using a microplate reader (Model 550, BioRad).

The affinity constant of mAbs was determined using a method developed by Beatty et al. (1987). Briefly, Lpp20-GST was coated on the ELISA plates at three different concentrations ($[Ag]$, $[Ag']$, and $[Ag'']$) followed by incubating with each mAb at different concentrations. The affinity constant of each mAb was calculated based upon the sigmoid curves representing the relationship between the concentration of mAb and the OD values. The antibody concentration at half maximal absorption for each curve was obtained by extrapolating the curves named $[Ab]$, $[Ab']$, and $[Ab'']$. The affinity constant was calculated using the following equation: $K_{aff} = (n - 1) / 2 (n[Ab'] - [Ab])$, where $n = Ag / Ag'$.

Using competitive and inhibition ELISA tests, the topology of the epitopes recognized by the mAbs was studied. Competitive tests compared the ability of the various unlabeled mAbs (competitors) to compete with the peroxidase-conjugated mAbs for binding to Lpp20 immobilized on ELISA plates, in which saturating amounts of unlabeled mAbs were added to plates coated with Lpp20 protein prior to the addition of labeled mAbs. Different concentrations of each competitor mAbs in PBS containing a constant concentration of a labeled mAb were co-incubated with immobilized Lpp20 for 40 min at 37 °C. Following extensive washing with PBS, the plates were developed with *O*-phenylenediamine dihydrochloride (Pierce Chemicals) as mentioned above. The percentage inhibition was finally estimated based on the following calculation: percentage inhibition = $[(OD \text{ without competitor}) - (OD \text{ with competitor}) / OD \text{ without competitor}] \times 100$. In inhibition experiments, labeled mAb was added following incubation of different concentrations of unlabeled mAbs with immobilized Lpp20.

2.4. Phage peptide library screening using a specific mAb as a target molecule

The Ph.D.-12 library was obtained from New England BioLabs Inc. They are combinatorial peptides fused to the minor coat protein (pIII) of M13 phage and consist of about 1.5×10^{13} pfu/ml electroporated sequences. Three rounds of biopanning were undertaken according to the manufacturer's instructions with some modifications. Briefly, a well was coated with 100 µg of mAb L001 in 0.1 M NaHCO₃ pH 8.6 and incubated overnight at 4 °C. The well was then washed 10 times with TBST (50 mM Tris, 150 mM NaCl, pH 7.5 containing 0.1% Tween-20), filled with 400 µl of blocking buffer (0.1 M NaHCO₃ pH 8.6, 5 mg/ml BSA and 0.02% NaN₃) and incubated for 1 h at 4 °C. In the first round of biopanning, 10 µl of phage (1.5×10^{11}) from the initial library in 100 µl of TBST was incubated with the well-bound mAb for 1 h at room temperature with gentle shaking. After repeated washes with TBST, the bound phages were then eluted from the well with 100 µl of elution buffer (0.2 M glycine-HCl, pH 2.2). After neutralization with 15 µl of 1 M Tris-HCl, pH 9.1, 1 µl of phage solution was taken for dilution with LB medium and the titer of the phages was determined. The remaining eluant was then amplified by infecting 20 ml of a 1/100 dilution of an overnight culture of *E. coli* ER2738, as recommended in the instruction manual. The culture was incubated for 4.5 h at 37 °C with vigorous shaking. The supernatants were obtained and precipitated with PEG/NaCl. The recovery of the screened phages was

measured by the following calculation: $\text{recovery} = \frac{\text{eluted phage}}{\text{input phage}} \times 100\%$.

In the second and third rounds of biopanning, the amplified phages from the preceding round were incubated with mAb L001 as described above. The procedures were then identical to the first round except in the second round the coated mAb concentration was 10 mg/l and the washing steps were carried out with 0.3% TBST. In the third round the coated mAb concentration was 1 mg/l and 0.5% TBST was used for the washing steps.

2.5. Binding specificity of the selected phage by ELISA and DNA sequencing

An ELISA plate was coated with 10 µg/ml mAb L001 by incubation at 4 °C overnight; BSA was also used for the coating to serve as a cross-reactivity control. Then after blocking with 5% BSA in TBS, the affinity selected phages were added to the wells and allowed to bind at 37 °C for 1 h. The phage library was set as the negative phage control and *E. coli* ER2738 culture supernatant as the blank control. After the unbound phages were removed with 5% TBST, the bound phages were detected by incubation with peroxidase labeled murine anti-M13 antibodies (Promega, USA). The bound peroxidase was determined by incubation with *O*-phenylenediamine dihydrochloride (Pierce Chemicals) in buffer (30 mM citrate, 70 mM Na₂HPO₄, and 0.02% H₂O₂, pH 5.5). After the reaction was stopped by the addition of 3 N HCl, the absorbance at 492 nm was measured using an ELISA plate reader (model 550, BioRad). All the assays were carried out in triplicate.

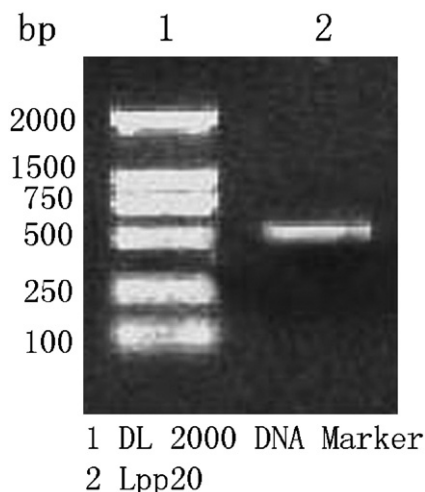


Fig. 1. Agarose gel electrophoresis of Lpp20 gene amplified by PCR. Lane 1, DNA marker; lane 2, amplified Lpp20 by PCR.

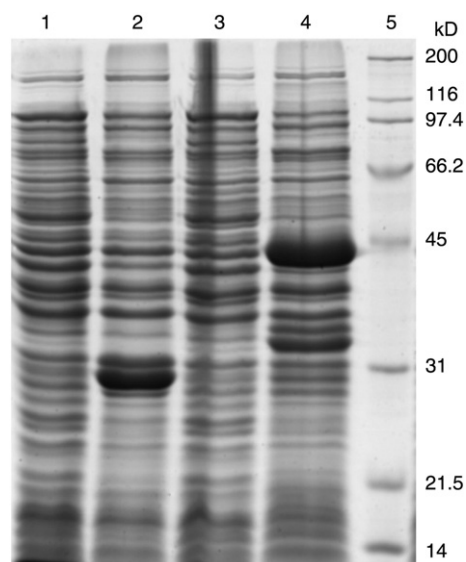


Fig. 2. SDS-PAGE analysis of expression of pGEX-4T-1/Lpp20 in *E. coli*. Lane 1, pGEX-4T-1 before induction; lane 2, pGEX-4T-1 after induction; lane 3, pGEX-4T-1/Lpp20 before induction; lane 4, pGEX-4T-1/Lpp20 after induction; lane 5, protein marker.

The phages from the third biopanning eluant were then cloned and amplified for DNA sequencing and immunoanalysis. The nucleotide sequence of the gene III insert was determined according to the manufacturer's instructions. The amino acid sequence of the peptides was deduced from the nucleotide sequence and compared with native Lpp20 protein sequence using DNAMAN software (Version 6.0). Alignments of the dodecapeptides with known Lpp20 protein sequences appearing more than three times in the selected phage clones were classified as the consensus sequence.

Competitive ELISA was used to analyze the binding specificities of the recombinant phages (C1 clone and C3 clone) to the screened mAb L001. The wells of the microtiter plates were first coated with Lpp20 protein and then incubated with the mAb L001 (0.5 µg) plus increasing amounts of the selected phages (10^9 – 10^{15} pfu) using wild type M13 phage as the negative control. The binding was monitored at A_{492} after reacting with the peroxidase-conjugated goat anti-mouse IgG (Sigma, USA). The inhibition ratio was calculated according to the formula: $\text{inhibition ratio} = \frac{[\text{OD}_{492}(\text{without phage}) - \text{OD}_{492}(\text{with phage})]}{\text{OD}_{492}(\text{without phage})} \times 100\%$.

2.6. Animal immunization and preparation of antisera

To evaluate the potential of the selected mimotopes as experimental vaccine candidates, two purified phages (clone C1 and clone C3) were used to immunize female

inbred specific-pathogen-free BALB/c mice through intraperitoneal administration. For each immunization, approximately 10^{12} pfu phages (in 50 μ l) were used and wild type M13 phage were used as the negative controls. Three mice were immunized with each sample and pre-immune serum (negative control) was obtained from three mice. Complement was inactivated by heating at 56 °C for 30 min before the antiserum titer assay. The mice were bled once (prebooster), then they were given boosters in the same manner. The immune mice were bled every 4 weeks after the booster immunization, and ELISA and Western blotting were used to test each serum sample for *H. pylori* Lpp20-specific antibody activity.

3. Results

3.1. Expression and purification of recombinant protein

To construct an expression *E. coli* strain of Lpp20 protein of *H. pylori*, the gene encoding Lpp20 protein was obtained by PCR amplification using the genomic DNA extracted from *H. pylori* as a template. The agarose gel electrophoresis analysis of the PCR product is shown in Fig. 1. A DNA fragment close to 525 bp was detected, which corresponded to the size of the Lpp20 gene. The DNA sequence of the hypothetical Lpp20 gene of *H. pylori* was determined by DNA sequencing

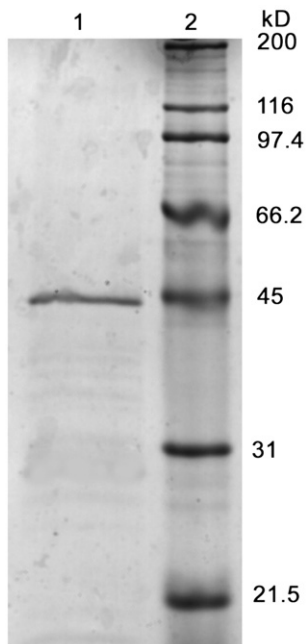


Fig. 3. SDS-PAGE analysis of purified Lpp20 protein by GST-affinity chromatography. 1, purified Lpp20; 2, protein marker.

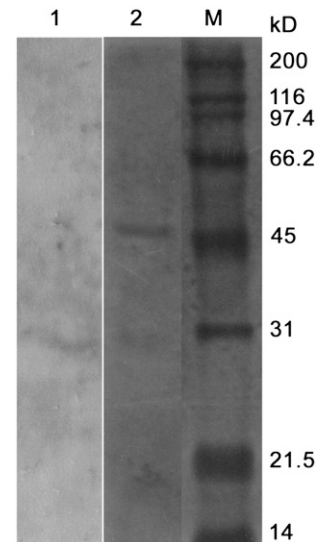


Fig. 4. Western blotting analysis of Lpp20 reactivity with the positive serum of *H. pylori*-infected patient. Lane 1, serum of a non-*H. pylori*-infected patient; lane 2, serum of patient infected with *H. pylori*; M, protein marker.

(data not shown) and submitted to GenBank (accession no. DQ106902). To facilitate the purification, Lpp20 was expressed as a GST fusion protein in *E. coli* Top10. The expression of the recombinant Lpp20-GST expressed in *E. coli* Top10, was examined by SDS-PAGE. A pronounced band with an approximate molecular weight of 48 kDa appeared in the supernatant of the cell lysate after the induction but not in the control cells, suggesting that the fusion protein was successfully expressed in the bacterial cells (Fig. 2). To use Lpp20-GST as the immunogen to generate monoclonal antibody against Lpp20, Lpp20-GST fusion protein was purified by glutathione-Sepharose 4B column chromatography. The purity of Lpp20-GST fusion protein was verified by SDS-PAGE analysis (shown in Fig. 3). The identity of the purified Lpp20-GST fusion protein was further confirmed by Western blotting. The serum of a *H. pylori*-infected patient specifically recognized the

Table 1

Enrichment of positive phage clones by panning over mAb L001 from Ph.D.-12 library

Cycle	Coating mAb (Mg/L)	Washing (% TBST)	Input phage (pfu)	Output phage (pfu)	Recovery rate (%)
I	100	0.1	1.5×10^{11}	8.4×10^4	5.6×10^{-7}
II	10	0.3	1.5×10^{11}	7.2×10^5	5.3×10^{-6}
III	1	0.5	1.5×10^{11}	3.0×10^6	2.0×10^{-5}

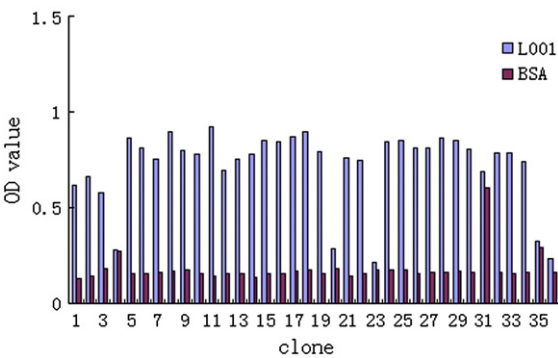


Fig. 5. Detection of clones from the Ph.D.-12 library with BSA cross-activity by ELISA. 1–34, clones; 35, the phage library; 36, *E. coli* ER2738 culture supernatant.

recombinant Lpp20-GST fusion protein, while the negative serum did not (shown in Fig. 4).

3.2. Production and characterization of anti-Lpp20 mAbs

Four hybridomas, L001–L004, were established by cell fusion. All mAbs were IgG1 (κ) and showed high specificity to Lpp20. The titer of the culture supernatant was 1/64 to 1/128 and the titer of ascites was 1/32,000 to 1/128,000. Three different concentrations of the antigen were used in the ELISA assay to calculate the K_{aff} values of each of the monoclonal anti-Lpp20 using the formula given in Section 2. The apparent affinity constants of the mAbs varied from $1 \times 10^8/\text{M}$ to $1 \times 10^{12}/\text{M}$. MAb L001 displayed the highest affinity of about $10^{12}/\text{M}$ (data not shown).

Competition studies using increasing concentrations of these four mAbs as inhibitors or competitors revealed that these four mAbs compete significantly with each other, indicating that the recognition epitopes of these four mAbs were the same or largely overlapped. The results of the inhibition assay were similar to those obtained by competition assay (data not shown).

Table 2
The sequences of Ph.D.-12 phage-displaying peptides from the recombinant phage clones randomly selected through biopanning

Clone no.	Sequence
Lpp20 protein	EKTRTV DASG KR (108–119aa)
C1–C2, C4–C6, C8–C10, C12–C14	SWPLYSDASGLG
C11, C15	VSLHDD ASG THR
C3	WLGESMISGWLY
C7	TKFTDAGDTSFN
C11	EHNDFPMYTWRP
C15	WPIEDMDLGPLYA

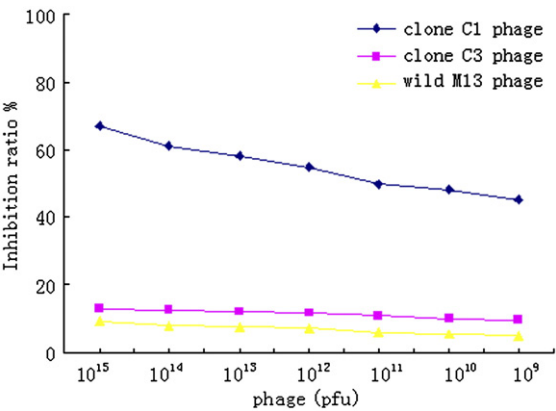


Fig. 6. Phage inhibition of mAb L001 binding to Lpp20 protein. Clone C1 phage (insert sequence SWPLYSDASGLG); lane 3, clone C3 phage (insert sequence WLGESMISGWLY).

3.3. Phage peptide library screening and characterization of the recombinant phages

In order to map the epitope of the Lpp20 antigen recognized by the mAb L001, the Ph.D.-12 library was screened with the purified mAb L001. After three rounds of biopanning, the phages bound to mAb were well enriched, as indicated by the increased recovery (Table 1). Then 34 clones of the phages after three rounds of biopanning were randomly selected to test for their immunoreactivity with mAb L001. Of the 34 clones, 30 clones were positively recognized by mAb L001 (shown in Fig. 5); BSA was used as negative control. The DNA sequences of 15 positive clones were determined and the deduced amino acid sequences of the corresponding peptides are shown in Table 2. There

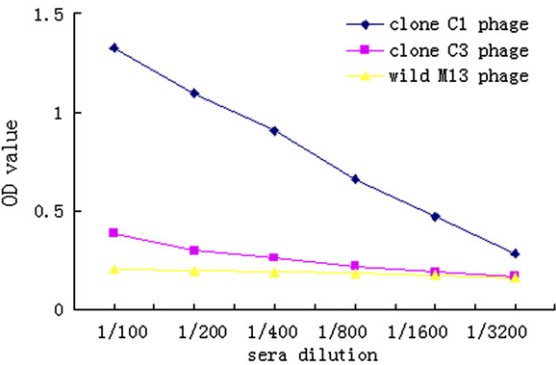


Fig. 7. Serum antibodies induced by selected phage clones. Serum samples were collected 4 weeks after intraperitoneal priming immunization. Triplicate ELISA measurements were performed for each sample using Lpp20 as coated protein.

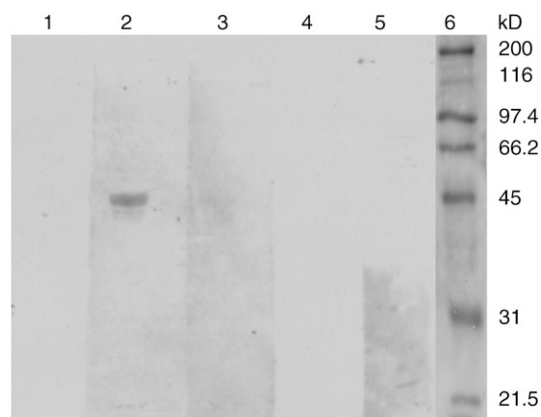


Fig. 8. Western blotting for recognition of antiserum induced by the selected phages with Lpp20 protein. Lane 1, pre-immune serum; lane 2, clone C1 phage (insert sequence SWPLYSDASGLG); lane 3, clone C3 phage (insert sequence WLGESMISGWLY); lane 4, wild type M13 phage; lane 5, TBS; lane 6, protein marker.

were six different peptides in these 15 phage clones. Comparison between the protein sequence of Lpp20 and these six dodecapeptides revealed that amino acid sequences DASG were conserved in Lpp20 (residues 114–117) and all positive phage clones. This result suggested that DASG was the core epitope of Lpp20 recognized by mAb L001. This notion was further confirmed by competitive ELISA assay. Pre-incubating mAb L001 with the recombinant phage clone C1 inhibited its binding with Lpp20 protein; pre-incubation with the control phage, for example phage clone C3, did not affect mAb's binding with Lpp20 (Fig. 6).

3.4. Immune response induced by the selected phage clones

To evaluate the immune responses in mice of the phage clones which comprised DASG epitopes, phage clone C1 and clone C3 were chosen to immunize BALB/c mice using intraperitoneal administration. As shown in Fig. 7, the serum of the mice immunized with phage clone C1 showed higher binding with pre-coated Lpp20 on an ELISA plate; the serum of the mice immunized with phage clone C3 showed similar low binding with Lpp20 as the controls (e.g. M13 phage).

The Lpp20 proteins recognized by the antisera (raised by phage clones and the controls) were verified further by Western blotting. The serum raised by clone C1 (lane 2) reacted specifically with the Lpp20 protein (48 kDa), while the pre-immune serum (lane 1), the serum induced by C3 clone (lane 3), by wild type M13 phage (lane 4) or TBS (lane 5) did not (shown in Fig. 8).

4. Discussion

The outer membrane is a continuous structure on the surface of gram-negative bacteria and, for bacterial pathogens, it is a potential target for the development of antibodies against bacterial infection. The functional and immunobiological characteristics of Lpp20 make it a good candidate for the development of a prophylactic vaccine for *H. pylori*. Mapping the epitope of Lpp20 is one of the essential steps for the development of antibodies against *H. pylori*.

Application of phage display technology to elucidate the essential chemical features of a pathogen's epitopes recognized by antibodies can provide important information about the molecular mechanisms of these diseases. The selected sets of peptides may also lead to future design of useful drugs for therapeutic and/or diagnostic purposes. In the field of vaccine design, phage-displayed mimotopes are under intensive investigation to make vaccine components that do not necessarily represent the structural equivalent of the original antigen, but provide functional images that could replace the original antigen for vaccine development (Bastien et al., 1997; Agadjanyan et al., 1997; De Berardinis et al., 2000). The main advantage of the phage display approach is that this method does not produce a consensus sequence of a large pool of peptides that share a common property, but rather yields a list of peptide sequences each of which is endowed with the same property. Aligning and comparing the amino acid sequences of the selected clones can then determine one or more consensus sequence. This approach enabled us to obtain the epitope profile of the microorganism in an efficient manner. The profiles not only reveal the important roles of each antigen, but also provide the epitope structures within the antigenic protein that are involved in the induction of the immune responses.

We first cloned and expressed Lpp20 of *H. pylori*, which was then used as immunogen to produce mAbs against this recombinant antigen by hybridoma technology. Four mAbs were obtained against Lpp20; mAb L001 had the highest affinity. We further identified the peptide sequence DASG as the epitope recognized by these mAbs through screening the Ph.D.-12 library, direct binding assay, competitive binding assay, DNA sequencing and sequence homology analysis. Furthermore, the phage clone which was recognized by mAb L001 can induce an immune response in mice. Therefore we considered that DASG may be the key amino acids forming an Lpp20 motif.

Phage display libraries cannot reach 100% homology with the known amino acid sequence of Lpp20. Many researchers have reached similar conclusions to

ours by using antibodies as a tool to screen the mimotopes from the phage epitope library. One reason to explain the difference between our mimotopes and the sequence of Lpp20 may be that the epitopes recognized by mAb could be either a linear epitope or a conformational epitope consisting of discontinuous amino acids. In the latter case, the discontinuous amino acids could get close to forming an epitope because of folding of the peptides.

It has been proposed that the eradication of pathogens depends on both the presence of serum antibody and an increase in cell-mediated immunity during *H. pylori* infection. Recombinant phages can display epitopes and also bear significant adjuvant activity (Willis et al., 1993). The selected phage clones were used to immunize mice and their potential as vaccine candidates was evaluated. The phagotopes also provide an easier and more economic way to prepare antigens and may be used in the current ELISA method for the diagnosis of *H. pylori*. Our findings showed that the phage-displayed epitope was able to mimic the immunological properties of the native epitope of *H. pylori* Lpp20. Furthermore, using the phage-displayed epitope as antigen, ELISA was able to detect the antibody in the serum of immunized animals.

In conclusion, the present study demonstrates the feasibility of identifying important mimotopes for vaccine development through screening of phage-displayed random peptide libraries with mAb. The results also indicate the potential of using phagotopes as alternative vaccine components. Comparison of mimotopes selected from a peptide library with mAb and the corresponding antigen sequence displayed on the phage may lead to a better understanding of the molecular mechanisms participating in the immune response and to the design of specific peptides for diagnostic purposes.

The hypothesis of our work is that an ideal protective immune response is based on a set of epitopes. Different combinations of epitopes could induce different characteristics of immune response. To build an immunogen by using epitopes we established a new strategy, called epitope-based vaccine design (EBVD). In light of EBVD, vaccine candidates are designed using natural epitopes identified by epitope mapping and mimotopes identified by phage display and combinational chemistry. In this paper, we report mimotopes identified from the Ph.D.-12 library through mAb of *H. pylori* Lpp20.

Acknowledgements

This work was supported by grants from Guangdong Natural Science Foundation Project (5004750),

National Key Development Projects (973 Project 2002CB513206), Guangzhou Major Science and Technology Project (2005Z1-E403) and we thank Dr Zhiping Wu (Department of Cell Biology, Cleveland Clinic, Cleveland, OH, USA) for revising the English of this manuscript.

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