Helicobacter pylori HopE and HopV porins present scarce expression among clinical isolates

Maritza Lienlaf, Juan Pablo Morales, María Inés Díaz, Rodrigo Díaz, Elsa Bruce, Freddy Siegel, Gloria León, Paul R Harris, Alejandro Venegas

AIM: To evaluate how widely Helicobacter pylori (H. pylori) HopE and HopV porins are expressed among Chilean isolates and how seroprevalent they are among infected patients in Chile.

METHODS: H. pylori hopE and hopV genes derived from strain CHCTX-1 were cloned by polymerase chain reaction (PCR), sequenced and expressed in Escherichia coli AD494 (DE3). Gel-purified porins were used to prepare polyclonal antibodies. The presence of both genes was tested by PCR in a collection of H. pylori clinical isolates and their expression was detected in lysates by immunoblotting. Immune responses against HopE, HopV and other H. pylori antigens in sera from infected and non-infected patients were tested by Western blotting using these sera as first antibody on recombinant H. pylori antigens.

RESULTS: PCR and Western blotting assays revealed that 60 and 82 out of 130 Chilean isolates carried hopE and hopV genes, respectively, but only 16 and 9, respectively, expressed these porins. IgG serum immunoreactivity evaluation of 69 H. pylori-infected patients revealed that HopE and HopV were infrequently recognized (8.7% and 10.1% respectively) compared to H. pylori VacA (68.1%) and CagA (59.5%) antigens.

Similar values were detected for IgA serum immunoreactivity against HopE (11.6%) and HopV (10.5%) although lower values for VacA (42%) and CagA (17.4%) were obtained when compared to the IgG response.

CONCLUSION: A scarce expression of HopE and HopV among Chilean isolates was found, in agreement with the infrequent seroconversion against these antigens when tested in infected Chilean patients.

Key words: Helicobacter pylori; Gene expression; HopE and HopV porins; Antigens; Immune response

Peer reviewers: Mario M D’Elios, Professor, University of Florence, viale Morgagni 85, Florence 50134, Italy; Andrew Day, Assistant Professor, University of Otago, Christchurch Hospital, Christchurch 8140, New Zealand
Helicobacter pylori (H. pylori) are Gram-negative, micro-aerophilic, spiral-shaped bacteria isolated from human gastric biopsies in 1983[1]. In order to survive in this aggressive environment, H. pylori are able to neutralize their close surrounding space by production of urease, which catalyzes the conversion of urea into ammonium and CO₂ raising pH close to neutral. In addition, to colonize the epithelium, this bacterium is able to bind to the epithelial cell surface, partially avoiding its removal by natural peristalsis or mucus renewal. These characteristics allow H. pylori to persist for decades.

H. pylori infection affects one half of the world population, roughly 73% in Chile[9], with higher prevalence as age increases. After several years of chronic gastric infection, approximately 10%-15% of infected patients develop severe gastrointestinal diseases such as chronic gastritis, peptic ulcer and gastric carcinoma[10]. In Chile, 5% of the infected population develops gastric cancer[11] and this malignancy is the second cause of death by cancer in the country.

H. pylori carries various virulence factors, and some may have potential as vaccine antigens. These factors may be grouped as: (1) colonization factors, which allow bacterial residence; (2) persistence factors which enable bacteria to accomplish an effective and lasting survival; and (3) disease inducing factors which cause adverse pathological effects on the gastric mucosa[8].

Based on a bioinformatics analysis of the H. pylori genome, a family of outer membrane proteins (OMPs) composed of 33 members has been identified[12]. These proteins are assembled into the outer membrane exposing, on the bacterial surface, small peptide loops which may act as epitopes to induce an immune response. This feature may be useful when selecting appropriate antigens for vaccine design. All these members contain an N-terminal signal peptide (processed by signal peptidase type I or II) that allows these proteins to cross the inner membrane on their way towards the outer membrane. The H. pylori OMPs form 2 families: the Hop members (21 proteins) and the Hor members (12 proteins). Hor proteins lack a characteristic N-terminal Hop motif[13]. Hop proteins have structural homology with the Escherichia coli (E. coli) outer membrane protein F (OmpF) porin[9]. Currently, 5 H. pylori Hop members (HopA, HopB, HopC, HopD and HopE) from strain 26695 have been characterized as porins using planar bilayer techniques[13,14] and some also behave as adhesins[15,16]. These properties make them attractive candidates as vaccine antigens. In fact, other bacterial porins from Salmonella, Pseudomonas, Chlamydia and Neisseria, have been found to be strong immunogens[17-19].

However, in the case of H. pylori, it has been suggested that not all the genes encoding OMPs may be functional at a given time. Some of these genes are under a control mechanism that operates by strand slippage during DNA replication or RNA repair. DNA polymerase slippage may easily add or remove nucleotides when DNA synthesis occurs in front of a homopolymeric tract or dinucleotide repeats at the template strand (i.e. polyG or polyC-A gene segments) causing mutations either at the promoter or at the coding region. This type of mutation may turn off or on some hop genes that may include these polynucleotide features. For instance, the hopC gene has been reported to carry a polyT tract (13 Ts in length) near the 5' end but hopA, hopB and hopE do not have long polyT tracts either at their coding regions or 5' upstream at the promoter regions. Gene switching will produce a change in the antigenic bacterial surface, a strategy that will distract the host immune system. For this reason, whether any H. pylori OMP would be considered as a vaccine antigen, omp genes containing long homopolymeric tracts or dinucleotide repeats should be avoided.

Regarding HopV and HopW, genetic heterogeneity in orthologous members of the Hop family among H. pylori strains has been described[16]. These new members were defined as part of the HopA/HopE family, because of their homology at the N-terminal sequence and the presence of 7 homologous domains in the C-terminus region. Regarding functional aspects, HopV and HopW have pore sizes similar to that of the E. coli OmpF porin[18] and HopE has been defined as the homolog to the E. coli OmpF porin[7].

Since the use of porins as antigens has been reported as successful[12-15], we decided to evaluate members of the H. pylori Hop family as putative antigen candidates for vaccine development by determining how widely they are expressed among Chilean H. pylori isolates and how often Chilean patients develop antibodies against them. A brief bioinformatic survey indicated that some genes of the Hop family had homopolymeric tracts or dinucleotide repeats in their coding sequences and promoter regions, with potential capability to promote strand slippage which may affect stability of gene expression[19]. Considering this aspect, only porin genes having single homopolymeric tracts or dinucleotide repeats no longer than 6 bases in their coding sequences were chosen as source of putative useful antigens for a vaccine. For this reason, among several OMP genes, only hopE and hopV sequences were selected for the present study.

MATERIALS AND METHODS

Bacterial strains and plasmid vectors
E. coli DH5α was used for polymerase chain reaction
(PCR) cloning, and E. coli BL21 (DE3), JM109 (DE3) and AD494(DE3)pLysS as host for porin expression assays. For cloning of PCR fragments plasmid pGEM-T Easy from Promega was used. For expression studies pET21a and pET21d (Novagen) were selected. H. pylori Chilean strain CHCTX-1 was used as DNA source for gene amplifications\(^1\). A collection of 130 H. pylori strains isolated from infected patients living in different Chilean cities was already available\(^2\).

**Bacterial cultures**

*E. coli* cells were grown overnight in LB media at 37°C with shaking. H. pylori strains were grown under 50 mL/L CO2 and 80% humidity in *Brucella* agar plates enriched with 5% horse blood cells and grown at 37°C for 2-3 d. *E. coli* strains were kept for short periods in LB plates at 4°C. Bacterial strains containing 14% glycerol were stored frozen for longer periods at -70°C.

**Plasmid purification, DNA manipulation and bacterial transformation**

Plasmids were usually detected by the “one step” method\(^3\), and purified by alkaline lysis method\(^4\). Restriction digestions, DNA ligations and plasmid dephosphorylations were done according to standard procedures\(^5\). Electroporation in 0.2 cm electrode separation thermocycler, with Gene Pulser\TM apparatus. Electroporative cells were grown overnight in LB media at 37°C with shaking. H. pylori strains were grown under 50 mL/L CO2 and 80% humidity in *Brucella* agar plates enriched with 5% horse blood cells and grown at 37°C for 2-3 d. *E. coli* strains were kept for short periods in LB plates at 4°C. Bacterial strains containing 14% glycerol were stored frozen for longer periods at -70°C.

**PCR assays**

Primers corresponding to the 5’ and 3’ ends as well as the internal sequences of the hopE and hopV genes (Table I) were designed based on *H. pylori* 26695 GenBank sequences. As templates, chromosomal DNA from the CHCTX-1 strain\(^6\) and from clinical isolates was prepared according to described protocols\(^7\). PCR reactions were carried out in a BioRad Mastercycler II thermocycler, with *Pfu* polymerase (Stratagene, CA, USA) or *Taq* polymerase (Promega, Madison WI, USA). Assays were done in 25 μL final volume following the manufacturer’s instructions. Gene amplicons were detected by 1% agarose gel electrophoresis. Other conditions were as previously described\(^8\). VacA alleles were determined by using primers and assay conditions described by Atherton et al\(^9\).

**Polyclonal antibodies against H. pylori OMPs**

According to standard procedures\(^10\), anti-HopE and anti-HopV rabbit antibodies were prepared. Proteins were obtained from *E. coli* clones expressing the *H. pylori* porins after separation by SDS-PAGE and purification from gel slices by electroelution as previously described\(^11\). Pathogen-free New Zealand adult female rabbits (approximately 1400 g in weight) were immunized with 250 μg of each porin dissolved in 2 mL of Tris-glycine buffer mixed (1:1) with complete Freund’s adjuvant. Two animals were used for each porin inoculation and 100 μL aliquots were applied subcutaneously in the back. This was followed by 3 boosters every 15 d.

**SDS-PAGE and Western blotting**

Lysates from clones expressing HopE and HopV were separated by polyacrylamide gels (12% or 15%) and run in minichambers according to Laemmli\(^12\). Western blotting were done as previously described\(^13\). As first antibody, patient serum (1:100 dilution) or rabbit anti-HopE, anti-HopV (1:1000 dilution) as second antibodies were used. As a second antibody for patient assays, goat anti-human IgA or anti-human IgG conjugated to peroxidase, were incubated (1:1000) overnight at 4°C. For anti-porin rabbit sera, a goat anti-rabbit peroxidase-conjugated antibody (1:1000) second antibody was used. To reduce cross reactions, rabbit antiserum and human antisera were adsorbed with sonicated lysates from *E. coli* AD494(DE3)pLysS/pET21d and BL21 (DE3), respectively. Human sera immunoblotting were done with lysates expressing HopE or HopV porins and products.

---

**Table 1** Primer sequences used for amplification and sequencing of *H. pylori* hopE and hopV genes

| Name   | 5’ - 3’ sequence                                                                 | Restriction sites |
|--------|-----------------------------------------------------------------------------------|------------------|
| HopV11 | GGGCTCTATGCCTCATGTTAATTTATGACAAACAGGAAATATGAAATGC                               | NdeI            |
| HopV12 | GGGCCCAATGCCAATCTCACTGTTATTG                                                     | BamHI           |
| HopE1  | GGGGCACTGAGTATTGATTAAGAGTTGTTTCTAGGG                                              | NcoI            |
| HopE2  | CCGGAAGCTTGAATGAGTTATGATTAACCTTATTAAGAAGGAGG                                     | HindIII         |
| HopE11 | GCAGATTGGTTGTTTGTAGAG                                                           | -               |
| HopE22 | ACCATATCAACATGATTTT                                                               | -               |
| HopV11 | GGCGTGCCCAGTTAGAATATCTTGG                                                       | -               |
| HopV22 | ACCATGTTTCTTATTCATC                                                              | -               |
| HopVIII | ATCGCCCTATATAGCCCCGGTGC                                                  | -               |
| pETT7d | TAATACGACTCATATAGGCC                                                               | -               |
| pETT7r | GCTAGTTATCGTACCCGCG                                                             | -               |

\(^1\)The restriction sites included in primer sequences and used for ligation to plasmid vectors are shown underlined; \(^2\)External primers used to clone porin genes; \(^3\)Internal primers used for gene sequencing and also for confirmatory PCR reactions for those cases in which primers derived from 5’ and 3’ gene ends failed to raise PCR products; \(^4\)Vector primers for 5’ and 3’ end gene sequencing.
derived from expression of *cagA* and *vacA* gene fragments cloned from strain CHCTX-1[19].

**Patient sera**

A sera panel from 69 infected patients (63 with gastritis, 6 with ulcers) recruited from the Universidad Católica de Chile Medical Center in Santiago, with signed consent, was available. Each patient's infected condition was defined by endoscopy, positive urease rapid test and detection of hematoxylin/eosin-stained curved bacteria on gastric tissue biopsies. Also, 8 non-infected patients were included in this study. The local ethics committee approved the protocol for this study.

**Immunoprecipitation of IgG from patient sera**

In order to obtain a cleaner IgA reaction in Western blotting assays using patient serum antibodies, protein G-plus-Agarose (Santa Cruz Biotechnology, catalogue #sc-2002) was utilized to first remove IgG from serum by immunoprecipitation. One hundred microliters of each serum without pre-adsorption treatment were incubated overnight with 200 μL of protein G-plus-agarose at 4°C with mild shaking. After sedimentation for 5 min at 2500 r/min and 4°C, the supernatant of each sample was used as a source of IgG-free serum.

**DNA sequencing**

DNA samples were previously purified by a commercial kit, and sequenced at our Sequencing Core Facility. T7 and internal primers for DNA sequencing are listed in Table 1.

**RESULTS**

**Cloning of porin genes derived from a Chilean *H. pylori* strain as putative antigens**

Selection for cloning and expression studies of the *hopE* and *hopV* genes were based on known gene sequences from strain *H. pylori* 26695. Since our study was focused on antigens obtained from local strains, *H. pylori* CHCTX-1 strain, a clinical isolate obtained from a Chilean patient[19] was selected as the DNA source for gene cloning in this study.

Cloning of HopE and HopV porin genes, including their signal peptide regions, was done by PCR. Primers and assay conditions are described in Table 1 and Methods, respectively. Amplicons from *hopE* and *hopV* genes were separated in a 1% agarose gel (Figure 1A), purified and treated with Taq polymerase and dATP to be ligated to pGEM-T. Recombinant plasmids were detected by insert release after EcoRI digestion and separation in 1% agarose gel electrophoresis. The *hopE* and *hopV* cloned inserts were subjected to *Nco I*-HindIII and *Nde I*-BamHI double digestions and ligated to pET21d and pET21a, respectively. As expected, fragments with sizes corresponding to these genes were observed. For expression purposes, plasmids were transferred to *E. coli* AD494(DE3)pLysS cells and visualized by the “one step method”[21]. Some clones containing plasmids with the *hopE* gene are displayed in Figure 1B. Purified plasmids were used for restriction digestions and also as DNA templates for PCR gene detection. *Nco I*-HindIII and *Nde I*-BamHI double digestions of plasmid DNA isolated from single clones were analyzed by agarose gel electrophoresis, and released inserts with sizes close to the expected ones (819 bp for *hopE* and 735 bp for *hopV*) were observed (Figure 1C).

**Expression of *H. pylori* HopE and HopV porin genes in *E. coli***

*E. coli* AD494(DE3)pLysS was able to express detectable amounts of HopE and HopV porins, as seen after SDS-PAGE and Coomassie blue staining (Figure 2A and B) and Western blotting assays (Figure 2C and D). Expression conditions were optimized by 5 h induction with 1 mmol/L isopropyl β-D-thiogalactoside

![Image of Figure 1 Cloning of H. pylori hopE and hopV genes. A: Polymerase chain reaction (PCR) amplification of hopE and hopV genes. Amplicons and plasmids were separated by 1% agarose gel electrophoresis. Lane Std: 1 kb DNA ladder standard. B: Detection of plasmids carrying the hopE gene. Lane 1: Strain AD494(DE3)pLysS with pET21d as control. Lanes 2, 3: Negative clones, lanes 4-7 plasmids carrying the hopE gene. C: Release of inserts carrying the hopE and hopV genes after *Nco I*-HindIII and *Nde I*-BamHI digestions respectively. Lane Std: 1 kb DNA ladder standard (Fermentas).](image-url)
Lienlaf M et al. Scarcie porin expression in *H. pylori* isolates

| kDa | Std | 1 | 2 | 3 | 4 | 5 | 6 |
|-----|-----|---|---|---|---|---|---|
| 85- |     |   |   |   |   |   |   |
| 47- |     |   |   |   |   |   |   |
| 34- |     |   |   |   |   |   |   |
| 26- |     |   |   |   |   |   |   |
| 19  | pET21d | - | + | + | - | - | - |
| 24  | pET21d-hopE | - | - | - | + | + | - |
| 1 mmol/L IPTG | - | + | + | - | - | + | - |
| 1 mmol/L IPTG | - | + | + | - | - | + | - |

**Figure 2** Expression of *H. pylori* *hopE* and *hopV* genes in *Escherichia coli* AD494(DE3)pLysS. Bacterial lysates were separated in 12%-15% PAGE-SDS gel, stained with Coomassie blue (A and B) or analyzed by Western blotting (C and D). Conditions for HopE expression are indicated below the figure (A, lanes 5 and 6; C, lane 2). Conditions for HopV expression are indicated under the figure (B, lanes 3 and 4; D, lane 2). Arrows indicate electrophoretic migration of these proteins. Lane Std: Prestained molecular weight marker in kDa (Fermentas).

(IPTG) for *hopE* and 0.8 mmol/L for *hopV* on previously saturated cultures. Protein sizes of 32 kDa for HopE and 28 kDa for HopV were observed. These porins displayed a certain amount of expression without IPTG induction, partially explained by the fact that the induction procedure was done on saturated cultures.

**Sequence analysis of *H. pylori* *hopE* and *hopV* genes**

The *hopE* (clone 1) and *hopV* (clone 13) gene sequences from the CHCTX-1 strain were obtained using external (T7 promoter and T7 terminator) as well as internal primers (Table 1) as described in Methods. Both *hopE* and *hopV* sequences were deposited at GenBank (accession numbers #EF635415 and #EF635416, respectively). As expected, these genes did not contain nonsense or frameshift mutations at their coding regions. Also, neither homopolymeric nor dinucleotide tracts longer than 6 nucleotides were found.

**Detection of *hopE* and *hopV* genes in Chilean clinical isolates and their expression**

From a collection of 240 clinical strains previously isolated, we selected 130 colonies (1 to 5 isolates per patient) as representatives from 6 Chilean cities: Iquique (IQ) in the North, Valparaiso (VA) and Santiago (SA) in the central region, Los Angeles (LA) and Valdivia (VL) in the South, and Punta Arenas (PA), the Southernmost city, to evaluate the distribution of strains carrying *hopE* and *hopV* genes and their expression throughout the country.

Detection of the genes was done by standard PCR. The amplicons were almost identical in size to those expected for *hopE* and *hopV* genes from strain 26695. Representative groups of isolates carrying *hopE* and *hopV* genes are shown in Figure 3A and B, respectively.

The *hopE* and *hopV* genes were detected in 46.9% (61 out of 130 strains) and 63.1% (82 out of 130) of the studied strains, respectively, and 40% (52 out of 130) of the strains revealed the presence of both genes simultaneously (Table 2). Among different cities, *hopE* and *hopV* gene contents varied between 30% and 69%. Curiously, *hopV* was frequently detected (69.2%) in strains from infected patients living in PA, the southernmost city. Patients from VL (mostly descendents from ancient aborigines) carried strains with a lower content (42.8%) of *hopV* gene. All PCR reactions were done at least twice using a pair of primers which bound to the gene ends. For those cases with negative amplification, additional assays using 2 primer combinations, including in each pair of primers one of the internal primers (Table 1), were performed. In most cases negative PCR reactions were confirmed and just a few strains showed positive PCR amplification only with pairs including internal primers, indicating that our initial estimation about the reduced presence of these genes in Chilean isolates was valid.

The positive results of HopE and HopV Western blotting expression assays in these isolates revealed no protein size variation, and selected results are displayed in Figure 3C.

Regarding porin expression, results showed that only 13.1% of the 130 strains expressed HopE and 6.9% expressed HopV. Altogether, 83.8% (109 out of 130 strains) did not express HopE or HopV porins either because of a lack of these genes, random inactivating gene mutations or gene silencing by the strand slippage mechanism (Table 2).

**Recognition of HopE and HopV porins by sera from infected patients**

In order to evaluate the capability of sera from Chilean *H. pylori*-infected patients to recognize recombinant *H. pylori* HopE and HopV porins, sera from 69 infected and 8 non-infected patients were tested. IgG and IgA serum antibodies against HopE and HopV antigens expressed as recombinant proteins in *E. coli* clones were tested using Western blotting assays on these bacterial lysates. VacA and CagA expressed similarly were used as immunodominant controls. The number of infected
phenotypes

| Genotypes [(E/V)] | No. of strains | Phenotypes [(E/V)] | No. of strains | No. of strains with different E/V phenotypes per city |
|---|---|---|---|---|
| (+/+) | 52 | (+/+) | 5 | IQ: 4 | VA: - | SA: - | LA: - | VL: - | PA: - |
| (+/-) | 12 | (+/-) | - | IQ: 4 | VA: - | SA: 5 | LA: 1 | VL: - | PA: 2 |
| (-/-) | 35 | (-/-) | 15 | IQ: 3 | VA: 3 | SA: 1 | LA: 7 | VL: 6 |
| (+/-) | 9 | (+/-) | - | IQ: 2 | VA: - | SA: - | LA: - | VL: - | PA: 5 |
| (-/-) | 26 | (-/-) | 2 | IQ: - | VA: - | SA: 4 | LA: - | VL: 2 | PA: 9 |
| (-/-) | 39 | (-/-) | 11 | IQ: 2 | VA: 11 | SA: 2 | LA: 7 | VL: 6 |
| Totals | 130 | Totals | 130 | IQ: 48 | VA: 54 | SA: 35 | LA: 26 | VL: 24 | PA: 26 |

1Presence of hopE (E) and/or hopV (V) genes in Chilean H. pylori isolates determined by PCR amplifications as described in Methods using purified DNA templates from single colonies collected from 69 patients of the indicated cities. (+): gene presence; (-): no detection; 2hopE and/or hopV expression assayed by Western blotting (see Methods). (+): detection; (-): no detection; 3Total number of strains with the assigned phenotype per city. IQ: Iquique; VA: Valparaiso; SA: Santiago; LA: Los Angeles; VL: Valdivia; PA: Punta Arenas. The number of patients per city was IQ = 21, VA = 4, SA = 12, LA = 3, VL = 5, PA = 15. Number of strains isolated per patient ranged between 1 and 5.

Figure 3  Detection of hopE and hopV genes and their expression in H. pylori clinical isolates from different Chilean cities. Amplicons were separated in 1% agarose gels. A: PCR detection of the hopE gene; B: PCR detection of the hopV gene. Arrows indicate migration of the respective gene fragments. Lane Std: 1 kb DNA ladder standard (Fermentas); Lane Std: Lambda DNA HindIII marker (Fermentas); C: Expression of HopV and HopE porins in H. pylori Chilean strains separated by 12% SDS-PAGE gels and detected by Western blots with respective polyclonal antibodies. Clinical isolates are indicated under the respective lanes. Std: Prestained molecular weight marker (Fermentas). The CHCTX-1 strain was included as a positive control.

Table 2  Number of strains presenting genotypes and corresponding phenotypes indicating the presence of hopE (E) and hopV (V) genes and their expression in 130 H. pylori strains isolated from infected patients from 6 Chilean cities

patient sera able to recognize these antigens are shown in Figure 4A. It was found that, as expected, IgG human antibodies more frequently recognized VacA (68.1% or 47 out of 69) and CagA (59.4%), but rarely recognized HopE (8.7%) and HopV (10.1%) porins. A similar distribution for HopE (11.6%) and HopV (10.5%) porins, but lower distribution values for VacA (42%) and CagA (17.4%) were found for IgA antibodies. The lower number of anti-HopE and anti-HopV reactive sera can be explained by the low proportion of H. pylori strains able to express these genes, being 13.1% (17/130) for HopE and 6.9% (9/130) for HopV (Table 2). Taken together, these results strongly suggest that H. pylori possesses a mechanism to switch on/off these OMP genes as a strategy to evade the host immune response.

In addition, considering that the immune response in children[31,32] could be quite different from that in adults[33], the IgA (Figure 4B) and IgG (Figure 4C) immune responses of the infected patients were plotted for 2 age groups: under 18 years of age and adults. It was noted that the number of sera with IgA and IgG responses against CagA antigen was significantly higher.
of the world’s population and induces strong serological and inflammatory responses in the host which persist during the entire life of the subject, rendering the host unable to eradicate infection. Knowledge of the most frequently recognized antigens in the infected population may contribute to an understanding of the bacterium survival strategy. In addition, this could also help to select appropriate antigens for vaccine design. The most extensively studied \textit{H. pylori} virulence factors as potential vaccine antigens are urease subunits\cite{34,35}, VacA and CagA\cite{36}, \textit{H. pylori} adhesin A\cite{37} and neutrophil-activating protein\cite{38,39}. However, results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.

PCR assays carried out on 130 Chilean isolates detected \textit{hopE} and \textit{hopV} genes in 46.9\% and 63.1\%, respectively. However, porin expression was infrequently detected in these strains (\textit{HopE} = 13.1\%, \textit{HopV} = 6.9\%), concurring with low seroprevalence of these porins among sera of infected patients, suggesting that these genes may be under DNA strand slippage control.

Infrequent detection of expression cannot be explained by low immunoreactivity of the rabbit antisera resulting from amino acid sequence diversity among strains, since these 2 porin sequences seem to be conserved. Results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.

PCR assays carried out on 130 Chilean isolates detected \textit{hopE} and \textit{hopV} genes in 46.9\% and 63.1\%, respectively. However, porin expression was infrequently detected in these strains (\textit{HopE} = 13.1\%, \textit{HopV} = 6.9\%), concurring with low seroprevalence of these porins among sera of infected patients, suggesting that these genes may be under DNA strand slippage control.

Infrequent detection of expression cannot be explained by low immunoreactivity of the rabbit antisera resulting from amino acid sequence diversity among strains, since these 2 porin sequences seem to be conserved. Results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.

PCR assays carried out on 130 Chilean isolates detected \textit{hopE} and \textit{hopV} genes in 46.9\% and 63.1\%, respectively. However, porin expression was infrequently detected in these strains (\textit{HopE} = 13.1\%, \textit{HopV} = 6.9\%), concurring with low seroprevalence of these porins among sera of infected patients, suggesting that these genes may be under DNA strand slippage control.

Infrequent detection of expression cannot be explained by low immunoreactivity of the rabbit antisera resulting from amino acid sequence diversity among strains, since these 2 porin sequences seem to be conserved. Results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.

PCR assays carried out on 130 Chilean isolates detected \textit{hopE} and \textit{hopV} genes in 46.9\% and 63.1\%, respectively. However, porin expression was infrequently detected in these strains (\textit{HopE} = 13.1\%, \textit{HopV} = 6.9\%), concurring with low seroprevalence of these porins among sera of infected patients, suggesting that these genes may be under DNA strand slippage control.

Infrequent detection of expression cannot be explained by low immunoreactivity of the rabbit antisera resulting from amino acid sequence diversity among strains, since these 2 porin sequences seem to be conserved. Results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.

PCR assays carried out on 130 Chilean isolates detected \textit{hopE} and \textit{hopV} genes in 46.9\% and 63.1\%, respectively. However, porin expression was infrequently detected in these strains (\textit{HopE} = 13.1\%, \textit{HopV} = 6.9\%), concurring with low seroprevalence of these porins among sera of infected patients, suggesting that these genes may be under DNA strand slippage control.

Infrequent detection of expression cannot be explained by low immunoreactivity of the rabbit antisera resulting from amino acid sequence diversity among strains, since these 2 porin sequences seem to be conserved. Results indicating reduction in colonization after oral vaccination of human subjects have been rather modest\cite{40,41}. As new antigens are needed, and there are few studies comparing porin genes among different \textit{H. pylori} strains, we have looked for \textit{H. pylori} porins suitable for vaccine development. The present report provides new information, particularly about Chilean strains, regarding porin expression among clinical isolates.

Folded porins have exposed loops at the bacterial surface which may induce a strong immune response. Porins have been proposed for the design of oral vaccines to eradicate \textit{H. pylori}\cite{42}. This pathogen has an extensive collection of OMPs with defined pore characteristics, such as the Hop group. However, some may display a unique on/off mechanism affecting gene expression, based on DNA strand slippage during replication. The instability of expression status makes antigen selection a very important issue. Antigens that will not be permanently expressed in most of the strains should be avoided, since they will provide a limited protection.
about 50% of the strains) and VacA (its gene is present in almost 100% of the strains) reacted with 59.4% and 68.1% of the IgG patient sera, respectively.

Regarding nucleotide sequence features, dinucleotide repeats in hopE and hopV sequences from the CHCTX-1 strain barely reached 5 nucleotides in length. However, they contained CCCCCC and TTTTTT tracts after codons 58 and 66, respectively. These findings, taken together with the low number of strains expressing these porins, and their low seroprevalence among Chilean patients, suggest that hopE and hopV may be under strand slippage gene control. Confirmation should be done by sequencing strains carrying silenced genes.

In H. pylori, at least 3 porin genes from the Hop family (hopZ, hopP and hopO) may be subjected to this on/off switching.

Another study showed a similar case: 3 different H. pylori strains re-isolated after Macaca rhesus infection lost expression of BabA adhesin which binds Lewis b antigen. These observations support the idea that H. pylori can modulate expression of some OMP genes. This feature provides an adaptive mechanism to avoid induction of a strong host immune response. This is supported by the large repertory of OMPs genes in the H. pylori genome. Functional redundancy of porins may explain emergence of mutations in these genes without affecting bacteria viability. It has been proposed that the role of such redundancy of outer membrane proteins is to sustain antigenic variation to support pathogen survival by evasion of the host immune response.

In spite of the low content of homopolymeric and dinucleotide repeats found in CHCTX-1 hopE and hopV genes, some strains may have switched these genes off but, in a few cases, expression could be restored by the same mechanism. This may restrict the use of these H. pylori OMPs as a single source of antigens for vaccine design. However, in order to provide a wider and stronger immune response, vaccines based on a mixture of H. pylori antigens with the inclusion of HopE and HopV should be considered.

ACKNOWLEDGMENTS

We thank Marco Olmos (Universidad Católica de Chile) for his careful review of the manuscript.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1: 1311-1315
2. Ferreccio C, Rollán A, Harris PR, Serrano C, Gederlini A, Margozzini P, Gonzalez C, Aguilara X, Venegas A, Jara A. Gastric cancer is related to early Helicobacter pylori infection in a high-prevalence country. Cancer Epidemiol Biomarkers Prev 2007; 16: 662-667
3. Sepulveda AR, Graham DY. Role of Helicobacter pylori in gastric carcinogenesis. Gastroenterol Clin North Am 2002; 31: 517-535
4. Suerbaum S, Josenhans C. Helicobacter pylori evolution and phenotypic diversification in a changing host. Nat Rev Microbiol 2007; 5: 441-452
5. Peek RM Jr, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002; 2: 28-37
6. Alm RA, Bina J, Andrews BM, Doig P, Hancock RE, Trust TJ. Comparative genomics of Helicobacter pylori: analysis of the outer membrane protein families. Infect Immun 2000; 68: 4155-4168
7. Exner MM, Doig P, Trust TJ, Hancock RE. Isolation and characterization of a family of porin proteins from Helicobacter pylori. Infect Immun 1995; 63: 1567-1572
Lienf M et al. Scarce porin expression in H. pylori isolates

8 Bond PJ, Sansom MS. The simulation approach to bacterial outer membrane proteins. Mol Membr Biol 2004; 21: 151-161

9 Doig P, Exner MM, Hancock RE, Trust TJ. Isolation and characterization of a conserved porin protein from Helicobacter pylori. J Bacteriol 1995; 177: 5447-5452

11 Ilver D, Arnvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T. Helicobacter pylori adhesin binding fucoylated histo-blood group antigens revealed by retagging. Science 1998; 279: 373-377

12 Peck B, Ortkamp M, Diehl KD, Hundt E, Knapp B. Conservation, localization and expression of HopZ, a protein involved in adhesion of Helicobacter pylori. Nucleic Acids Res 1999; 27: 3325-3333

11 Secundino I, Lopez-Macias C, Cervantes-Barragan L, Gil-Cruz C, Rios-Sarabia N, Pastelin-Palacios R, Villasen-Keever MA, Becker I, Puente JL, Calva E, Isibasi A. Salmonella porins induce a sustained, lifelong specific bactericidal antibody memory response. Immunochemistry 2006; 117: 59-70

13 Worgall S, Krause A, Rivara M, Hee KK, Vintayen EV, Andersen LP. Inflammation, immunity, and Helicobacter pylori. J Bacteriol 2004; 186: 6537-6547

15 Toropainen M, Saarinen L, Vidarsson G, Kåby H. Protection by meningococcal outer membrane protein PorA-specific antibodies and a serogroup B capsular polysaccharide-specific antibody in complement-deficient and C6-deficient infant rats. Infect Immun 2006; 74: 2803-2808

16 Peck B, Ortkamp M, Nau U, Niederweis M, Hundt E, Knapp B. Characterization of four members of a multigene family encoding outer membrane proteins of Helicobacter pylori and their potential for vaccination. Microbes Infect 2001; 3: 171-179

17 Bina J, Bains M, Hancock RE. Functional expression in Escherichia coli and membrane topology of porin HopE, a member of a large family of conserved proteins in Helicobacter pylori. J Bacteriol 2000; 182: 2370-2375

18 Salanit R, Linz B, Suerbaum S, Saunders NJ. The diversity within an expanded and redefined repertoire of phase-variable genes in Helicobacter pylori. Microbiology 2004; 150: 813-830

19 Müller I, Medina-Selby A, Palacios JL, Martinez P, Opazo P, Bruce E, Mancilla M, Valenzuela P, Yudilevich A, Venegas A. Cloning and comparison of ten gene sequences of a Chilean H. pylori strain with other H. pylori strains revealed higher variability for VacA and CagA virulence factors. Biol Res 2002; 35: 67-84

20 Díaz MI, Valdivia A, Martinez P, Palacios JL, Harris P, Novales J, Garrido E, Valderrama D, Shilling C, Kirberg A, Hebel E, Fierro J, Klapp G, Leon G, Klapp G, Venegas A. Helicobacter pylori vacA s1a and s1b alleles from clinical isolates from different regions of Chile show a distinct geographic distribution. World J Gastroenterol 2005; 11: 6366-6372

21 Beuken E, Vink C, Bruggeman CA. One-step procedure for screening recombinant plasmids by size. Biotechniques 1998; 24: 748-750

22 Sambrook J, Fritsch E, Maniatis T. Molecular Cloning. A laboratory manual. 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989

23 Miller JF. Bacterial transformation by electroporation. Methods Enzymol 1994; 235: 375-385

24 Owen R, Bickle J. Isolation of H. pylori genomic DNA and restriction analysis. In: Clayton CL, Mobley LH, editors. Helicobacter pylori protocols. Methods in Molecular Medicine. Totowa, New Jersey: Humana Press, 1997: 81-88

25 Serrano C, Diaz MI, Valdivia A, Godoy A, Peta A, Rollan A, Kirberg A, Hebel E, Fierro J, Klapp G, Venegas A, Harris PR. Relationship between Helicobacter pylori virulence factors and regulatory cytokines as predictors of clinical outcome. Microbes Infect 2007; 9: 428-434

26 Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995; 270: 17771-17777

27 Cooper HM, Paterson Y. Production of Antibodies. In: Coligan JE, Kruisbeek AM, Margulies DH, Sherbach EM, Strober W, editors. Current protocols in immunology. New York, NY: John Wiley and Sons Inc, 1995; 2:4-2.4.9

28 Every D, Green RS. Purification of individual proteinase isozymes from Bacteroides nodosus by use of polycrylamide gel electrophoresis, a fluorogenic substrate detection system, and a simple electrophoresis apparatus. Anal Biochem 1982; 119: 82-85

29 Laemmli UK, Beguin P, Gujer-Kellenberger G. A factor preventing the major high protein of bacteriophage T4 from random aggregation. J Mol Biol 1970; 47: 69-85

30 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350-4354

31 Campbell DI, Pearce MS, Parker L, Thomas JE. IgG subclass responses in childhood Helicobacter pylori duodenal ulcer: evidence of T-helper cell type 2 responses. Helicobacter 2004; 9: 289-292

32 Campbell DI, Pearce MS, Parker L, Thomas JE, Sullivan PB, Dale A. Immunoglobulin G subclass responses to Helicobacter pylori vary with age in populations with different levels of risk of gastric carcinoma. Clin Diagn Lab Immunol 2004; 11: 631-633

33 D’Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S, Del Prete G, T helper 1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease. J Immunol 1997; 158: 962-967

34 Kreiss C, Buclin T, Cosma M, Corhês-Thuelaz I, Michetti P. Safety of oral immunisation with recombinant urease in patients with Helicobacter pylori infection. Lancet 1999; 347: 1630-1631

35 Michetti P, Kreiss C, Kottloff KL, Porta N, Banco J, Bachmann D, Herranz M, Saldinger PF, Corhês-Thuelaz I, Losonsky G, Nichols R, Simon J, Stölte M, Ackerman S, Monath TP, Blum AL. Oral immunization with urease and Escherichia coli heat-labile enterotoxin is safe and immunogenic in Helicobacter pylori-infected adults. Gastroenterology 1999; 116: 804-812

36 Yan J, Mao YF, Shao ZX. Frequencies of the expression of main protein antigens from Helicobacter pylori isolates and production of specific serum antibodies in infected patients. World J Gastroenterol 2005; 11: 421-425

37 Nyström J, Svennerholm AM. Oral immunization with HpAa affords therapeutic protective immunity against H. pylori that is reflected by specific mucosal immune responses. Vaccine 2007; 25: 2591-2598

38 Malfhertheiner P, Schwittke V, Rosenkranz B, Kaufmann SH, Ulrichs T, Novicki D, Contorni M, Peppoloni S, Berti D, Tornese D, Gannu J, Palla E, Rappuoli R, Scharrschmidt BF, Del Giudice G. Safety and immunogenicity of an intramuscular Helicobacter pylori vaccine in noninfected volunteers: a phase I study. Gastroenterology 2008; 135: 787-795

39 D’Elios MM, Andersen LP. Inflammation, immunity, and...
vaccines for Helicobacter pylori. Helicobacter 2009; 14 Suppl 1: 21-28

40 Lee CK. Vaccination against Helicobacter pylori in non-human primate models and humans. Scand J Immunol 2001; 53: 437-442.

41 Bumann D, Metzger WG, Mansouri E, Palme O, Wendland M, Hurwitz R, Haas G, Aebischer T, von Specht BU, Meyer TF. Safety and immunogenicity of live recombinant Salmonella enterica serovar Typhi Ty21a expressing urease A and B from Helicobacter pylori in human volunteers. Vaccine 2001; 20: 845-852.

42 Yamaoka Y, Kita M, Kodama T, Imamura S, Ohno T, Sawai N, Ishimaru A, Imanishi J, Graham DY. Helicobacter pylori infection in mice: Role of outer membrane proteins in colonization and inflammation. Gastroenterology 2002; 123: 1992-2004.

43 Solnick JV, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M. Modification of Helicobacter pylori outer membrane protein expression during experimental infection of rhesus macaques. Proc Natl Acad Sci USA 2004; 101: 2106-2111.

44 Deitsch KW, Moxon ER, Wellems TE. Shared themes of antigenic variation and virulence in bacterial, protozoal, and fungal infections. Microbiol Mol Biol Rev 1997; 61: 281-293.