Deletion of Helicobacter pylori vacuolating cytotoxin gene by introduction of directed mutagenesis

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INTRODUCTION

Helicobacter pylori (H pylori) is a Gram-negative bacterium that colonizes the gastric mucosa of humans[13], and plays an important role in pathogenesis of chronic gastritis, peptic ulcers, gastric adenocarcinomas, and gastric mucosa-associated lymphoid tissue lymphomas[14-16]. Leunk et al first reported in 1988 that cell-free supernatants from H pylori broth cultures induced striking vacuolar degeneration when added to cultured gastric cells co-cultured with cell-free supernatants from H pylori wild strain or the mutant.

METHODS AND RESULTS: A clone containing kanamycin resistance gene used for homologous recombination was constructed in a directional cloning procedure into pBluescript II SK, and then transformed into vacA+ H pylori by electroporation. Colonies growing on the selective media containing kanamycin were harvested for chromosomal DNA extraction, and the allelic exchange was determined by polymerase chain reactions and sequencing. Loss of vacuolating activity of the vacA-knockout strain was confirmed by examining the gastric cells co-cultured with cell-free supernatants from H pylori wild strain or the mutant.

CONCLUSION: We constructed a vacA-knockout strain of H pylori through direct mutagenesis, which creates an important precondition for the future research on virulence comparison with gene expression analysis.

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Abstract

AIM: To construct a vacA-knockout Helicobacter pylori mutant strain, whose only difference from the wild strain is its disrupted vacA gene.

METHODS AND RESULTS: A clone containing kanamycin resistance gene used for homologous recombination was constructed in a directional cloning procedure into pBluescript II SK, and then transformed into vacA+ H pylori by electroporation. Colonies growing on the selective media containing kanamycin were harvested for chromosomal DNA extraction, and the allelic exchange was determined by polymerase chain reactions and sequencing. Loss of vacuolating activity of the vacA-knockout strain was confirmed by examining the gastric cells co-cultured with cell-free supernatants from H pylori wild strain or the mutant.

CONCLUSION: We constructed a vacA-knockout strain of H pylori through direct mutagenesis, which creates an important precondition for the future research on virulence comparison with gene expression analysis.

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INTRODUCTION

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The gene encoding the vaculating cytotoxin has been cloned from an H pylori isolate, and termed vacA[18]. Analysis of the nucleotide sequence of the vacA open reading frame (ORF) suggested that vacA encoded a 139-kDa protoxin that has three functional domains: a 33-amino-acid N-terminal signal sequence, a mature cytotoxin domain (approximately 87kDa), and a cleaved C-terminal domain (approximately 50kDa)[19,20]. VacA could induce vacuole formation from the cell cytosol, as determined by transfection of epithelial cells with a plasmid encoding the complete 95-kDa domain of VacA[21]. These vacuoles are acidic, and their membrane contains the vacuolar ATPase proton pump and the small GTP-binding protein rab7. Therefore, they have been suggested to arise from late compartments of the endocytic pathway[22].

Over the past decade, there has been considerable effort directing toward understanding the molecular mechanisms underlying VacA action. But till now, little is known about the mechanisms of vacuole formation and other effects of VacA. In this study, using the technique of direct mutagenesis to disrupt vacA gene, we constructed a vacA-knockout H pylori mutant strain for the further research on virulence comparison between the H pylori wild strain and the mutant.

MATERIALS AND METHODS

Bacterial strain and growth conditions

H pylori NCTC 11638 as a gift from Dr. Tong Shi (Shanghai Institute of Digestive Diseases) was cultured routinely on brain heart infusion (BHI) agar plates with 5 % sheep blood in an environment containing 6 % CO2 at 37 °C. For the preparation of cell-free supernatants from H pylori broth cultures, H pylori was cultured in BHI broth+10 % fetal bovine serum (FBS) in an environment containing 6 % CO2 at 37 °C with agitation (200 rpm) for 48 h. The cultures were centrifuged (15 000 g, 30 min, 4 °C) and filtrated with a 0.2 µm syringe filter.

Disruption of vacA gene

The strategy for disruption of vacA gene by direct mutagenesis is shown in Figure 1, and genetic techniques involved were described as follows.

DNA isolation To isolate chromosomal DNA, H pylori cells were lysed in lysis buffer (10 mM Tris·HCl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.5 % (w/v) SDS, 20 µg/ml DNase free pancreatic RNase) and then, protease K (Sangon, Shanghai, China) was added in to a final concentration of 100 µg/ml. The lysate was incubated in a water bath at 50 °C for 3 h. Then, the solution was cooled to room temperature, and mixed with an equal volume of phenol equilibrated with 0.1 M Tris·HCl (pH 8.0). The two phases were separated by centrifugation at 5 000 g for 15 min at room temperature, and the aqueous phase was extracted with phenol twice again. Afterwards, 0.2 volume of 10 M ammonium acetate and 2 volume of ethanol were added to the aqueous phase. The precipitate was cultured in BHI broth+10 % fetal bovine serum (FBS) in an environment containing 6 % CO2 at 37 °C with agitation (200 rpm) for 48 h. The cultures were centrifuged (15 000 g, 30 min, 4 °C) and filtrated with a 0.2 µm syringe filter.

Polymerase chain reactions (PCR) PCR was carried out in 100 µl volume containing 100 ng of genomic DNA, 1 U of Ex Taq (Takara), 50 pmol of each primer, and 10 nmol of each deoxynucleoside triphosphate in a standard buffer. Oligonucleotide primers (5'-CGTGGAAATCTTATTACTTTAGC-3' and 5'-TGATGCTGACTAATGCTCCT-3')
were used to amplify a 1.7 kb product from \textit{H pylori} NCTC 11 638. Primers for amplifying kanamycin resistance gene (\textit{kanR}) and two fragments flanking \textit{kanR}, \textit{LA} and \textit{RA}, were designed as shown in Table 1.

**Gel purification and enzyme digestion**  PCR products were electrophoresed and excised from a 1 % agarose gel, purified using a Qiaquick gel extraction kit (Qiagen, Hilden, Germany), and digested with corresponding restriction enzymes (Promega, Madison, USA) depending on different restriction sites.

**Cloning of different DNA fragments**  Purified PCR products for sequencing were cloned into \textit{pGEM-T} vector (Promega). Fragments \textit{kanR}, \textit{LA}, and \textit{RA} with different restriction sites on both sides were digested with corresponding endonucleases (Promega), and then cloned into \textit{pBluescript SK} II digested with the same enzymes.

**Sequencing**  Every clone was sequenced with the ABI DNA sequencer (Bioasia Biotechnology Company, Shanghai, China).

**\textit{H pylori} DNA transformation by electroporation**  \textit{H pylori} NCTC 11 638 cells were transformed with plasmid \textit{pLKR} by electroporation, and kanamycin-resistant (\textit{Km} \textit{r}) transformants were selected by a method similar to that described by Clayton \textit{et al} \cite{15}. Briefly, \textit{H pylori} cultured on plates were scraped and suspended in 30 ml cold double-distilled water. Cells were harvested by centrifugation at 4 360 \textit{g} at 4 \textdegree C for 5 min, and the pellet was suspended in 20 ml of cold 10 % glycerol. The cells were centrifuged once, and resuspended in 2 ml ice-cold 10 % glycerol. Plasmid DNA (1 \textmu g in 5 \textmu l TE buffer) was mixed with 0.2 ml cell suspension. The mixture was added to a prechilled (-20 \textdegree C) 0.2 cm electroporation cuvet (Bio-Rad, Hercules, USA), and subjected to single-pulse electroporation of initial voltage 2.5 kV, 25 \textmu F and 600\Omega in parallel. The sample was transferred onto a cold plate and incubated for 12 h at 37 \textdegree C. Then the cells were inoculated onto selective media with 30 \mu g/mL kanamycin, followed by incubation for 4 d to allow the growth of transformants.

**Cell culture and detection of vacuole formation**  Cells of gastric cancer cell line SGC7901 as a gift from Jie Yang (Department of Cell Biology, Shanghai Second Medical University) were grown in DMEM (GIBCO-BRL, Gaithersburg, USA) supplemented with 10 \% FBS, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 \mu g/mL streptomycin in a humidified 5 \% CO\textsubscript{2} atmosphere. SGC7901 cells were co-cultured with cell-free supernatants from \textit{H pylori} NCTC11638 wild strain or mutant strain for 12 h, and then observed by contrast microscopy.

![Diagram](attachment:image.png)

**Figure 1** The strategy for disruption of \textit{vacA} gene by directed mutagenesis. LA and RA were PCR-amplified from \textit{H pylori} NCTC 11638 genomic DNA, and the kanamycin resistance gene, from \textit{pEGFP-N1}. PCR products with different restriction sites on both sides were digested with corresponding endonucleases, and then cloned into \textit{pBluescript SK} digested with the same enzymes. Because there is an EcoR II site in RA, pLA and \textit{pkm} \textit{r} were firstly joined together, resulting in pLK, which then was joined with pRA, resulting in pLKR. The plasmid pLKR was transformed into \textit{H. pylori} NCTC 11638, where the \textit{Km} \textit{r} marked mutation was introduced into the genome by homologous recombination, resulting in the \textit{vacA} \textit{Km} \textit{r} mutant strain.
Table 1: Primers for amplifying \( km' \), LA and RA

| Primer | Sequence (5' to 3') | Site | Coordinates |
|--------|---------------------|------|-------------|
| la1    | GGGGTACCCTTTTGAGCCTTTAGTT | Kpn I | cysS bp36-54 |
| la2    | CGGAATTCTCTTTTTTTTTTAAAC | EcoR I | HPU07145 bp382-400 |
| km'1   | CGGAATTCTATGAAACAAGAGTTTGAGTG | EcoR I | pEGFP-N1 bp 2629-2649 |
| km'2   | CGGGATCCTCAAGAAACTCGTCAAAG | BamHI | pEGFP-N1 bp3406-3423 |
| ra1    | CGGGATCCTACGCCCTCTGGGTTCTC | BamHI | HPU07145 bp 437-454 |
| ra2    | GCTCTAGACACCCACTTGATTATCTTACTCT | Xba I | HPU07145 bp 1786-1807 |

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AGTTGAAAAG AAGGCCGATA TTATGGGTG CGGGCCTAGCG GTGTATGATG
101 AGCGTCAATTGGGCGATGCA TTGAAAGCGT TTTAGAAAAA AATATCGCTT
151 CGACCGCTTG GAAAGGCCGGT CATAGAACAT CTCAAAGCGG GCCAGATCTG
201 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
251 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG
301 ATGTTTATTT ATGATACCAA ATTAAAACA AAAGTCCCTT TTGAGCCTTT
351 AGTTGAAAAA AAGGCGAATA TTTATGTGTG CGGGCCTACG GTGTATGATG
401 ATCAGGCTCCT TAAATGGGTG ATATTGCGTC GACCTTTGGCC
451 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
501 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG
551 ATGTTTATTT ATGATACCAA ATTAAAACA AAAGTCCCTT TTGAGCCTTT
601 AGTTGAAAAA AAGGCGAATA TTTATGTGTG CGGGCCTACG GTGTATGATG
651 CGACCGCTTG GAAAGGCCGGT CATAGAACAT CTCAAAGCGG GCCAGATCTG
701 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
751 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG
801 ATGTTTATTT ATGATACCAA ATTAAAACA AAAGTCCCTT TTGAGCCTTT
851 AGTTGAAAAA AAGGCGAATA TTTATGTGTG CGGGCCTACG GTGTATGATG
901 ATCAGGCTCCT TAAATGGGTG ATATTGCGTC GACCTTTGGCC
951 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
1001 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG
1051 ATGTTTATTT ATGATACCAA ATTAAAACA AAAGTCCCTT TTGAGCCTTT
1101 AGTTGAAAAA AAGGCGAATA TTTATGTGTG CGGGCCTACG GTGTATGATG
1151 CGACCGCTTG GAAAGGCCGGT CATAGAACAT CTCAAAGCGG GCCAGATCTG
1201 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
1251 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG
1301 ATGTTTATTT ATGATACCAA ATTAAAACA AAAGTCCCTT TTGAGCCTTT
1351 AGTTGAAAAA AAGGCGAATA TTTATGTGTG CGGGCCTACG GTGTATGATG
1401 ATCAGGCTCCT TAAATGGGTG ATATTGCGTC GACCTTTGGCC
1451 TTAACCTGCCG CAACTGAAAT SACGGGCGTC GACCTTTGGCC
1501 GCTGACGGTCA TCTAGCAGT GCCTTATGAG GCGGAGATCTG

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151 GCAACAGAAA TTTCTAGTC TAAAGTGCGCA CCGTTTGGCC AAAATCGGTT
1601 TTATGTTGAGAAT CCAGGGAGAT CTATGATGGA ACAAGATGGG TTGCACGGCAG
1651 GCTTTCACGGCGCTTGGGTTG GAGAGCTTATCA CTGCTATGGC TCGGCCAGAA
1701 CAGACAATGCGTGTCTGCTG TCGCCGCGTG TTCCGGCGTG CAGCGCGAGG

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Figure 2 Nucleotide sequence of cysS gene and the downstream sequence amplified from the vacA Km\(^r\) mutant H. pylori. The 1398 bp cysS ORF and the 795 bp Km\(^r\) ORF are shown. Primers la1, la2, ra1, ra2, and rs for amplifying LA, RA, and ASm are indicated. -35 signal, -10 signal, and rbs of vacA gene serving Km\(^r\) gene in the mutant strain are also shown.
RESULTS

Upstream sequence close to vacA gene

Genome of NCTC 11638 was not completely sequenced, and the upstream portion close to vacA gene going to be used in the mutagenesis technique was not published in GeneBank. Therefore, the upstream sequences close to vacA gene of 26695 and 399, whose genomes were completely sequenced and published, were aligned and searched for conservative sequences. Then a 1.7-kb product was PCR-amplified from H pylori NCTC11638 DNA and sequenced. The sequencing result showed the complete cysS (cysteinyl-tRNA synthetase) gene of NCTC 11638 (Figure 2).

Cloning of pLKR for transforming H pylori

As shown in Figure 1, LA which contains the H pylori vacA promoter and RA were amplified from genomic DNA of NCTC11638, while kanR gene which has no promoter was amplified from the plasmid pEGFP-N1 (Clontech, Palo Alto, USA). PCR-products LA, kanR and RA, with restriction sites incorporated at the termini, were joined together in a directional cloning procedure into pBluescript II SK, resulting in pLKR.

Construction of vacA-knockout H pylori mutant strain

pLKR which is unable to replicate in H pylori, was introduced into H pylori NCTC11638 by electroporation. After 4 d of growth, five Km' single colonies were isolated. To determine whether vacA had been disrupted in the transformed strains through allelic exchange, DNA isolated from H pylori NCTC11638 wild-type strain and the Km' mutant strain were PCR-amplified with the primers la1 and rs (5' -CGCCTTATCCTGCCAAAGCATAGC-3') annealing to H pylori NCTC 11638 vacA at bp 1808 to 1832 flanking ru2 (Figures 1 and 2). A 3.8-kb product consistent with the expected size was PCR-amplified from Km' mutant strain, as compared with a 3.0-kb product amplified from wild strain (Figure 3), suggesting a substitution of Km' gene for a short fragment of vacA gene by homologous recombination between plasmid and chromosomal sequences. The sequencing result of the 3.8-kb product confirmed the occurrence of allelic exchange (Figure 2).

DISCUSSION

VacA, produced by pathogenic strains of H pylori, was a major virulence factor in pathogenesis of gastroduodenal ulcers[8,16]. VacA induced the formation of membrane-delimited vacuoles in intoxicated cells[7], and showed many other effects on cellular functions and viability, such as causing mitochondrial depolarization[17], inducing apoptosis in gastric cells[19], affecting or interacting with various components of cytoskeleton to cause actin rearrangements[19], and even disorganizing microtubular network[20]. To study VacA mechanism of action, we have tried to get purified VacA used as single virulence determinant, to study its effect on the expression profile of eukaryocyte. However, like Manetti et al[21], we did not successfully get the expressed VacA as a functional recombinant protein in E. coli, probably due to its incorrect fold. We have also considered letting VacA directly expressed in the cytosol to induce vacuole formation. In our experiments, vacuoles were induced in only 10 % of cells transfected with plasmids expressing VacA, because the efficiency of the transfection method was relatively low. In addition, when VacA acts outside the cells, the pathway by which it interacts with the cells is quite different from that when the protein is produced in the cytosol. Under natural conditions, association of VacA with the eukaryotic cell surface was the first step in the intoxication of cells[22]. The initial interaction of VacA with target cells was through high-affinity cell surface receptors, and this interaction was necessary for its biologic activity[23,24]. A 250 kDa receptor protein tyrosine phosphatase (RPTP)β served as a receptor for VacA on AZ521 cells, and another protein, p140, was also commonly detected in VacA-susceptible cells[25,26]. Increased binding of acid- or alkali-activated VacA to RPTPβ may alter its activity and possibly accelerates or inhibits dephosphorylation of tyrosine on cytosolic proteins. Moreover, VacA acting outside the cells...
is a kind of exogenous antigen, having different pathways of processing and presentation from that of VacA expressed in the cytosol as an endogenous antigen. All of these processes may affect gene expression of the host cells.

Direct mutagenesis was probably the most useful technique for assessing the contribution to virulence of specific bacterial gene products[27]. In our study, vacA gene encoding vacuolating cytotoxin that has been identified by conventional biochemical means was disrupted by gene replacement. This technique requires a means for introducing DNA into the pathogen, as well as suitable selective markers and an inherent capacity for homologous recombination. In previous studies, the Km³ determinant often came from Campylobacter coli[28]. Here we introduced a simple method to get the Km³ gene from commercialized plasmids such as pEGFP-N1. Coding sequence of the gene without a promoter was PCR-amplified from pEGFP-N1 and ligated downstream with the promoter of H pylori vacA gene. Upon insertion into chromosomal DNA of H pylori through homologous recombination, this gene could be efficiently transcribed because the vacA promoter was recognized by H pylori transcriptional machinery, introducing kanamycin resistance characteristics into H pylori. Due to the stop codon of Km³, vacA would not be translated at all although most of the sequences still existed. The results of PCR and sequencing confirmed the occurrence of allelic exchange. Therefore, using the direct mutagenesis technique, we obtained the isogenic mutant strain of H pylori, which differed from the wild strain only in that the vacA gene was knocked out. Through co-culture of cell-free supernatants from the wild or mutant H pylori strain with gastric cells, loss of vacuolating activity of the vacA-knockout strain was confirmed. These results clearly show that VacA is an indispensable toxin secreted by H pylori for the induction of vacuole formation.

Such kind of technique has been used to yield vacA mutant H pylori[29-32]. But no further experiment has been done to compare the virulence between the mutant and the parental strain. On the other hand, microarray analysis has been used in several studies to screen gene expression profiles in gastric epithelial cells induced by H pylori[30-32]. Our group has also analyzed different expression profiles of gastric cancer cells co-cultured with supernatants of VacA⁺ or VacA⁻ H pylori isolates. However, VacA has not been used as a single virulence determinant to stimulate host cells, thus one can not determine which virulent factors result in the alteration of the expression. In this study, we successfully constructed the vacA mutant strain, using the direct mutagenesis technique, which creates an important precondition for the further research on virulence comparison with gene expression analysis.

REFERENCES

1 Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clin M icrob Rev 1997; 10: 720-741
2 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1: 1311-1315
3 Graham DY, Lew GM, Klein PD, Evans DG, Evans DJ Jr, Saeed ZA, Malaty HM. Effect of treatment of Helicobacter pylori infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. Ann Intern Med 1992; 116: 705-708
4 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orenstrech N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991; 325: 1227-1231
5 Forman D, Nellwell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BM J 1991; 302: 1302-1305
6 Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H pylori infection with gastric carcinoma: a Meta analysis. World J Gastroenterol 2001; 7: 801-804
7 Leunk RD, Johnson PT, David BC, Kraft-WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. J Med M icrob 1988; 26: 93-99
8 Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from Helicobacter pylori. J Biol Chem 1992; 267: 10570-10575
9 Cover TL, Tummuru MK, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. J Biol Chem 1994; 269: 10566-10573
10 Schmitt W. Haas R. Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. M ol M icrob 1994; 12: 307-319
11 Hou P, Tu ZX, Xu GM, Gong YF, Ji XH, Li ZS. Helicobacter pylori vacA genotypes and cagA status and their relationship to associated diseases. World J Gastroenterol 2000; 6: 605-607
12 De Bernard M, Arico B, Papini E, Rizzuto R, Grandi G, Rappuoli R, Montecucco C. Helicobacter pylori toxin VacA induces vacuole formation by acting in the cell cytosol. M ol M icrob 1997; 26: 655-674
13 Papini E, de Bernard M, Milia E, Bugnoli M, Zerial M, Rappuoli R, Montecucco C. Cellular vacuole induced by Helicobacter pylori originate from late endosomal compartments. Proc Natl Acad Sci U S A 1994; 91: 9789-9794
14 Sambrook J, Russell DW. Molecular Cloning-A Laboratory Manual. 3th ed, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press 2001; Vol 1: 6.4-6.11
15 Clayton CL, M obley HLT. Methods in Molecular Medicine, Helicobacter pylori Protocols. 1st ed, Totowa: Humana Press Inc 1997: 145-152
16 Cover TL. The vacuolating cytotoxins of Helicobacter pylori. M ol M icrob 1997; 20: 241-246
17 K imura M, Goto S, Wada A, Yahiro K, Niidome T, Hatakeyama T, Aoyagi H, Hirayama T, Kondo T. V acuolating cytotoxin purified from Helicobacter pylori causes mitochondrial damage in human gastric cells. M ol M icrob 1999; 26: 45-52
18 Kuck D, Kolmerrer B, Iking-Konert C, Krammer PH, Stremmel W, Rudi J. V acuolating cytotoxin of Helicobacter pylori induces apoptosis in the human gastric epithelial cell line AGS. Infect Immun 2001; 69: 5080-5087
19 Ashorn M, Cantel F, Mayou K, Megraud F. Cytoskeletal rearrangements induced by Helicobacter pylori strains in epithelial cell culture: possible role of the cytotoxin. D ig Dis Sci 2002; 45: 1774-1780
20 Pai R, Cover TL, Tarnawski A. S. Helicobacter pylori vacuolating cytotoxin (VacA) disorganizes the cytoskeletal architecture of gastric epithelial cells. Biochim Biophys Res Commun 1999; 262: 245-250
21 Manetti R, Massari P, Burroni D, de Bernard M, Marchini A, Orlandi R, Papini E, Montecucco C, Rappuoli R, Telford JL. Helicobacter pylori cytotoxins: importance of native conformation for induction of neutralizing antibodies. Infect Immun 1995; 63: 4476-4480
22 Papini E, Zoratti M, Cover TL. In search of the Helicobacter pylori VacA mechanism of action. Toxicon 2001; 39: 1757-1767
23 Garner JA, Cover TL. Binding and internalization of the Helicobacter pylori vacuolating cytotoxin by epithelial cells. Infect Immun 1996; 64: 4197-4203
24 Massari P, Manetti R, Burroni D, Nuti S, Norais N, Rappuoli R, Telford JL. Binding of the Helicobacter pylori vacuolating cytotoxin to target cells. Infect Immun 1998; 66: 3981-3984
25 Yahiro K, Niidome T, K imura M, Hatakeyama T, Aoyagi H, Kurazono H, Imagawa K, Wada A, M osson J, Hirayama T. Activation of Helicobacter pylori VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphataseβ. J Biol Chem 1999; 274: 36693-36699
26 Yahiro K, Niidome T, Hatakeyama T, Aoyagi H, Kurazono H, Padilla PI, Wada A, Hirayama T. Helicobacter pylori vacuolating cytotoxin binds to the 140-kDa protein in human gastric cancer cell lines, AZ-521 and AGS. Biochim Biophys Res Commun 1997; 238: 629-632
27 Henderson B, Wilson M, McNab R, Lax AJ. Cellular Microbiology: Bacteria-host interactions in health and disease. Hoboken: John Wiley Sons Ltd 1999: 163-188
28 Copass M, Grandi G, Rappuoli R. Introduction of unmarked mutations in the Helicobacter pylori vacA gene with a sucrose sensitivity marker. Infect Immun 1997; 65: 1949-1952
29 Burroni D, Lupetti P, Pagliaccia C, Reyrat JM, Dallai R, Rappuoli R, Telford JL. Deletion of the major proteolytic site of the Helicobacter pylori cytotoxin does not influence toxin activity but favors assembly of the toxin into hexameric structures. Infect Immun 1998; 66: 5547-5550
30 Sepulveda AR, Tao H, Carloni E, Sepulveda J, Graham DY, Peterson LE. Screening of gene expression profiles in gastric epithelial cells induced by Helicobacter pylori using microarray analysis. Aliment Pharmacol Ther 2002; 16(Suppl 2): 145-157
31 Cox JM, Clayton CL, Tomita T, Wallace DM, Robinson PA, Crabtree JE. cDNA array analysis of cag pathogenicity island-associated Helicobacter pylori epithelial cell response genes. Infect Immun 2003; 69: 6970-6980
32 Maeda S, Otsuka M, Hirata Y, Mitsuno Y, Yoshida H, Shiratori Y, Masuho Y, Muramatsu M, Seki N, Omata M. cDNA microarray analysis of Helicobacter pylori-mediated alteration of gene expression in gastric cancer cells. Biochem Biophys Res Commun 2003; 284: 443-449

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