

A VACCINE COMPOSITION COMPRISING *HELICOBACTER PYLORI* FLAGELLIN POLYPEPTIDE

## TECHNICAL FIELD

- 5 The present invention relates to polypeptides and vaccine compositions for inducing a protective immune response to *Helicobacter pylori* infection. The invention furthermore relates to the use of a *Helicobacter pylori* polypeptides in the manufacture of compositions for the treatment or prophylaxis of *Helicobacter pylori* infection.

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## BACKGROUND ART

*Helicobacter pylori*

- 15 The gram-negative bacterium *Helicobacter pylori* is an important human pathogen, involved in several gastroduodenal diseases. Colonization of gastric epithelium by the bacterium leads to active inflammation and progressive chronic gastritis, with a greatly enhanced risk of progression to peptic ulcer disease.
- 20 In order to colonize the gastric mucosa, *H. pylori* uses a number of virulence factors. Such virulence factors comprise several adhesins, with which the bacterium associates with the mucus and/or binds to epithelial cells; ureases which helps to neutralize the acid environment; and proteolytic enzymes which makes the mucus more fluid. In addition, motility is essential for sustained colonization in the gastric mucosa as shown by the
- 25 inability of *Helicobacter* mutants lacking flagella to colonize the gastric mucosa (Akopyants et al. Infection & Immunity 63(1):116-21, 1995).

- Despite a strong apparent host immune response to *H. pylori*, with production of both local (mucosal) as well as systemic antibodies, the pathogen persists in the gastric mucosa,
- 30 normally for the life of the host. The reason for this is probably that the spontaneously

induced immune-response is inadequate or directed towards the wrong epitopes of the antigens.

### *Flagellins*

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Flagella are organelles which are involved in locomotion of bacterial cells and are found primarily on the surface of rod and spiral shaped bacteria. The filaments of flagella are made up of specific proteins, known as flagellins.

10 A vaccine, derived from *E. coli* flagella, for the protection against *E. coli* infections, is disclosed in EP 0413378. Vaccines where flagellin proteins have been used as adjuvants, i.e. compounds which are mixed with the immunogen to increase the immune response, are disclosed in WO 88/01873 and WO 89/10967.

15 Antigenic compositions comprising flagella for use in diagnostic kits for detection of *Campylobacter (Helicobacter) pylori* are disclosed in US 5,459,041. However, there is no mention of the use of *Helicobacter pylori* flagellin in inducing a protective immune response to *Helicobacter pylori* infection.

20 *Helicobacter pylori* flagellin (H.p. flagellin) is a structural protein of the *H. pylori* flagella. *Helicobacter pylori* flagellin consists of two subunits, FlaA and FlaB. The *flaA* and *flaB* gene of *Helicobacter* have been cloned (see Leying, H. et al., Molecular Microbiology 6(19): 2863-74, 1992). Mutation experiments have shown that FlaA is absolutely essential for the motility, whereas some motility is preserved in the absence of FlaB (Josenhans, C.  
25 et al., J. Bacteriology 177(11): 3010-3020, 1995). In all *Helicobacter* species living in the stomach, the flagella appears to be totally covered by a flagellar sheath (Geis, G. et al., J. Med. Microbiol. 38(5):371-377, 1993.) The purpose of this sheet is unknown, but it could be important for survival in the hostile gastric environment.

30 Early studies showed that deeper located *Helicobacter pylori* in the human stomach can be covered with sIgA and more rarely with IgM and IgG (Wyatt, J.I. et al., J. Clin. Pathol. 39:

863-870, 1986). Reasons for this could be that the antibodies are not reacting with any functionally essential sites and/or that cellular immunity does not work in the mucosa. It is known that the complement system does not function in the gastric mucosa. Antigens giving rise to protective mucosal immunity are usually presented to mucosal surfaces with M-cells. The gastric mucosa has no or very few such antigen recognizing cells and thus the antigen detection probably is poor. In order to get the appropriate protective immune response, the right antigens have to be presented at the right site.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1:

Therapeutic oral immunization in *H. pylori* infected mice. Results are given as CFU (colony-forming units) of *H. pylori* in 25 mm<sup>2</sup> gastric mucosa (geometric mean values). Abbreviations: CT, cholera toxin; Mp, membrane proteins.

Fig. 2:

Serum antibody titers against *H. pylori* flagellin in infected control animals and following immunization with *H. pylori* flagellin in infected animals. Values expressed as relative OD values.

Fig. 3:

Effect of monoclonal antibodies raised against flagellin on the colonization of *H. pylori* in the mouse stomach. Antibodies were mixed with *H. pylori* prior to inoculation of mice (n = 10 in all groups). Geometric mean values of CFU is displayed.

Fig. 4:

Gastric colonization with *H. pylori* expressed as geometrical means in antrum and corpus mucosa. Animals receiving recombinant FlaA + CT showed significant decreased colonization in the antrum. 3/10 animals had no bacteria in antrum. \*p<0.05 (Wilcoxon-Mann-Whittney sign rank test)

Fig. 5:

Serum IgG response to *H. pylori* infection and to immunization with recombinant FlaA + CT. Data shown are mean $\pm$ SEM. ELISA plates were coated with membrane protein from strain 244 (m.p. 244) or with rFlaA. All animals showed immune response to *H. pylori* infection. Only animals given rFlaA + CT had IgG antibodies against FlaA.

Fig. 6:

Mucosal IgA antibodies against FlaA in Stomach and Duodenum mucosa. Data shown as mean $\pm$ SEM.

#### DISCLOSURE OF THE INVENTION

Natural infection of *H. pylori* in man will induce a systemic immune response to flagellin. In spite of this no protection or clearance of the infection is obtained. It has now surprisingly been found that a significant suppression and eradication of *H. pylori* is seen in infected mice when purified flagellin is given. In addition, it has been found that when *H. pylori* is incubated with a monoclonal antibody to H.p. flagellin, prior to inoculation with the bacteria in mice, infection in the animals is completely prevented.

On basis of these findings, the following conclusions are made:

- Part of the *H. pylori* flagella is exposed to antibody attack and thus not totally covered by the flagellar sheath.
- The *H. pylori* flagellar protein acts as a strong and consistent antigen when it, in a purified form, is presented to a mucosal surface.
- Purified *H. pylori* flagellin will stimulate a competent local immune response capable of significantly decreasing or eradicating *H. pylori* colonization of the gastric mucosa.
- The mechanism of antibody binding to the flagella is potent, since pre-binding of monoclonal antibodies to *H. pylori* flagellin completely inhibits colonization of *H. pylori*.

Consequently, the present invention is directed to a polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide, or a modified form of the said polypeptide retaining functionally equivalent antigenicity, for use in inducing a protective immune response to *Helicobacter pylori* infection.

The term "*Helicobacter pylori* flagellin polypeptide" should be understood as a polypeptide forming part of the basic structure of *Helicobacter pylori* flagella. In preferred forms of the invention, the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaA or FlaB.

The term "functionally equivalent antigenicity" is to be understood as the ability to induce a systemic and mucosal immune response while decreasing the number of *H. pylori* cells associated with the gastric mucosa. The skilled person will be able to identify modified forms of the polypeptide retaining functionally equivalent antigenicity, by use of known methods, such as epitope mapping with *in vivo* induced antibodies.

The term "protective immune response" is intended to mean an immune response which makes the composition suitable for therapeutic and/or prophylactic purposes.

In another important aspect, the invention provides a vaccine composition for inducing a protective immune response to *Helicobacter pylori* infection, comprising an immunogenically effective amount of a polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide, optionally together with a pharmaceutically acceptable carrier or diluent. In preferred forms of the invention, the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaA or FlaB.

In the present context the term "immunologically effective amount" is intended to mean an amount which elicits a significant protective *Helicobacter pylori* response, which will suppress or eradicate a *H. pylori* infection in an infected mammal or prevent the infection in a susceptible mammal. Typically an immunologically effective amount will comprise

approximately 1 mg to 1000 mg, preferably approximately 10 mg to 100 mg, of *H. pylori* antigen for oral administration, or approximately less than 100 mg for parenteral administration.

- 5 The vaccine composition comprises optionally in addition to a pharmaceutically acceptable carrier or diluent one or more other immunologically active antigens for prophylactic or therapeutic use. Physiologically acceptable carriers and diluents are well known to those skilled in the art and include e.g. phosphate buffered saline (PBS), or, in the case of oral vaccines,  $\text{HCO}_3^-$  based formulations or enterically coated powder formulations.

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- The vaccine composition can optionally include or be administered together with acid secretion inhibitors, preferably proton pump inhibitors (PPIs), e.g. omeprazole. The vaccine can be formulated in known delivery systems such as liposomes, ISCOMs, cochleates, etc. (see e.g. Rabinovich et al. (1994) Science 265, 1401-1404) or be attached  
15 to or incorporated into polymer microspheres of degradable or non-degradable nature. The antigens could be associated with live attenuated bacteria, viruses or phages or with killed vectors of the same kind. The antigens can be chemically or genetically coupled to carrier proteins of inert or adjuvantic types (i.e. Cholera B subunit). Consequently, the invention provides in a further preferred aspect a vaccine composition according to above, in addition  
20 comprising an adjuvant, such as a pharmaceutically acceptable form of cholera toxin. Such pharmaceutically acceptable forms of cholera toxin are known in the art, e.g. from Rappuoli et al. (1995) Int. Arch. Allergy & Immunol. 108(4), 327-333; and Dickinson et al. (1995) Infection and Immunity 63(5), 1617-1623.

- 25 A vaccine composition according to the invention can be used for both therapeutic and prophylactic purposes. In this context the term "prophylactic purpose" means to induce an immune response which will protect against future infection by *Helicobacter pylori*, while the term "therapeutic purpose" means to induce an immune response which can suppress or eradicate an existing *Helicobacter pylori* infections.

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The vaccine composition according to the invention is preferably administered to any mammalian mucosa exemplified by the buccal, the nasal, the tonsillar, the gastric, the intestinal (small and large intestine), the rectal and the vaginal mucosa. The mucosal vaccines can be given together with for the purpose appropriate adjuvants. The vaccine can  
5 also be given parenterally, by the subcutaneous, intracutaneous or intramuscular route, optionally together with the appropriate adjuvant.

Yet another aspect of the invention is the use of a polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide in the manufacture of compositions for the  
10 treatment or prophylaxis of *Helicobacter pylori* infection; and in particular in the manufacture of a vaccine for use in eliciting a protective immune response against *Helicobacter pylori*. In preferred forms of the invention, the said polypeptide comprises *Helicobacter pylori* flagellin, or the *Helicobacter pylori* polypeptide FlaA or FlaB.

15 In a further aspect, the invention provides a method of eliciting in a mammal, including man, a protective immune response against *Helicobacter pylori* infection, said method comprising the step of administering to the said mammal an immunologically effective amount of a vaccine composition as defined above.

20 In preferred forms of the above aspects of the invention, the *Helicobacter pylori* FlaA subunit has substantially the amino acid sequence set forth as SEQ ID NO: 2 in the Sequence Listing, or is a modified form thereof retaining functionally equivalent antigenicity. The *Helicobacter pylori* FlaB subunit has substantially the amino acid sequence set forth as SEQ ID NO: 4 in the Sequence Listing, or is a modified form thereof  
25 retaining functionally equivalent antigenicity.

It is thus to be understood that the definition of the *Helicobacter pylori* FlaA and FlaB polypeptides is not to be limited strictly to polypeptides with amino acid sequences identical with SEQ ID NO: 2 or 4, respectively, in the Sequence Listing. Rather the  
30 invention encompasses polypeptides carrying modifications like substitutions, small deletions, insertions or inversions, which polypeptides nevertheless have substantially the

biological activities of the *Helicobacter pylori* FlaA and FlaB polypeptides and are retaining functionally equivalent antigenicity. Included in the definition of the *Helicobacter pylori* FlaA and FlaB polypeptides are consequently polypeptides, the amino acid sequence of which is at least 90% homologous, preferably at least 95% homologous, with the amino acid sequence set forth as SEQ ID NO: 2 and 4 in the Sequence Listing.

## EXPERIMENTAL METHODS

### (a) Purification of flagellin from *Helicobacter pylori* flagella

*H. pylori* was grown on 100 horse blood plates for 2-3 days in a microaerophilic atmosphere. The cells were harvested by scraping off and suspending bacteria from the plates in cold PBS, ca 40 ml in total.

Flagellin was prepared by a modification of the method described by Kostrzynska et al. (J. Bacteriol. 173, 937-946, 1991) as outlined below.

Flagella were removed by homogenization for 2 x 30 sec with an Ultra-Thurrax mixer (13.500 rpm). Deflagellated cells were removed by centrifugation for 1 h, +4°C at 18.000 x g. The flagella were then pelleted by ultracentrifugation for 1 h at 100.000 x g. The resulting pellets were resuspended in 4 ml of 20 mM Tris-HCl buffer, pH 7.8, containing 20 mM CaCl<sub>2</sub> and 160 µl of trypsin (25 mg/ml) was added. The flagella were then incubated for 80 min at +37°C. The reaction was terminated by adding 40 µl of trypsin inhibitor (25 mg/ml).

CsCl<sub>2</sub> (4.9 g) was dissolved in the trypsin treated sample and 8.1 ml H<sub>2</sub>O was added. The defraction index was adjusted to 1.27 g/cm<sup>3</sup>. The samples were centrifuged for 20 h at 180.000 x g in a swing-out rotor. The visible band was collected from the gradient, dialyzed over night with 20 mM phosphate buffer, pH 7.0. The optical density at 280 and 310 nm was measured and the protein content was calculated. The material was analyzed

by SDS-PAGE. After staining with Coomassie Brilliant blue, two bands corresponding to the flagellin subunits FlaA and FlaB were seen.

(b) Production of *Helicobacter pylori* flagellin monoclonal antibodies.

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Female SPF BALB/c mice were purchased from Bomholt Breeding centre (Denmark). They were kept in ordinary makrolon cages with free supply of water and food. The animals were 4-6 weeks old at arrival.

- 10 Purified flagellins from *H. pylori* strain E50 were used to immunize BALB/c mice for production of monoclonal antibodies as described previously by De St. Groth and Scheidegger (J. Immunol. Methods. 35, 1-21, 1980). Briefly, 5-10 µg purified flagellin was injected i.p. and i.v. in Balb/c mice with and without Freund's complete adjuvant 5 times during 109 days. Spleen cells were prepared and fused with myeloma cells by standard  
15 procedures.

The resulting hybrids were analyzed by ELISA as described (Lopez-Vidal et al. (1988) J. Clin. Microbiol. 26, 1967-1972) using 5 µg/ml of purified flagellins for coating. The antibody-secreting hybridomas having the highest ELISA titers were cloned and expanded.  
20 Culture fluids from established hybridomas were harvested and frozen at -20°C and the corresponding antibody-producing cells were frozen in liquid nitrogen for long-term storage. The monoclonal anti-flagellin antibody used in subsequent studies was denoted HP50F-48:13;1.

25 (c) Isolation of the *Helicobacter pylori* *flaA* and *flaB* genes

The *flaA* and *flaB* genes were cloned from a *Helicobacter pylori* genomic library, constructed from *Helicobacter pylori* CCUG 17874 DNA in Lambda Zap Express.

- 30 A genomic clone containing the entire sequence of the *flaA* was isolated using two probes obtained from PCR amplification of the 5' - and 3' -regions of the gene. Two synthetic

oligonucleotides complementary to the 5'-region, and two complementary to the 3'-region of the previously cloned *Helicobacter pylori flaA* gene (Leying H. et al. (1992) Mol. Microbiol. 6(19), 2863-2874), were synthesized and used for PCR-amplification of the probes. The probes were <sup>32</sup>P-labelled by Amershams Megaprime labelling system.

- 5 Approximately 30,000 individual plaques were analysed. One plaque hybridizing to the 5'- and 3'-regions of the gene was isolated. *In vivo* excision of the pBK-CMV plasmid from the Zap Express vector was performed and the resulting plasmid was designated pS947. The complete sequence of the *flaA* gene (SEQ ID NO: 1) was determined using PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing (PERKIN ELMER).

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A genomic clone containing the entire sequence of the *flaB* gene was isolated using two probes obtained from PCR amplification of the 5'- and 3'-region of the gene. Two synthetic oligonucleotides complementary to the 5'-region, and two complementary to the 3'-region of the previously cloned *H. pylori flaB* gene (Suerbaum S. et al. (1993) J.

- 15 Bacteriol. 175, 3278-3288) were synthesized and used for PCR-amplification of the probes. The probes were <sup>32</sup>P-labelled by Amershams Megaprime labelling system. Approximately 30,000 individual plaques were analysed. One plaque hybridizing to the 5'- and 3'-regions of the gene was isolated. *In vivo* excision of the pBK-CMV plasmid from the Zap Express vector was performed and the resulting plasmid was designated pS948. The complete  
20 sequence of the *flaB* gene (SEQ ID NO: 3) was determined using PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing (PERKIN ELMER) according to the manufacturers protocol.

(d) Construction of expression vector for recombinant *Helicobacter pylori* FlaA protein

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In order to produce high levels of the recombinant FlaA protein (SEQ ID NO: 2), the expression vector pS997 was constructed. The vector contained the *Helicobacter pylori flaA* gene under control of the T7 promoter.

- 30 In order to change restriction sites in the 3'-end of the *flaA* gene, two synthetic oligonucleotides (SEQ ID NO: 5 and SEQ ID NO: 6) for PCR amplification were

synthesized. The plasmid pS947 (flaA-pBK-CMV) was used as a template for the PCR amplification. PCR amplification was performed and the amplified fragment was digested with *Xma*I and *Pst*I generating a 339 bp fragment. This fragment was cloned into pUC19, the constructed plasmid was designated pS989. The sequence of the construct was confirmed by sequencing as above.

To generate convenient restriction sites for the 5'-end of the *flaA* gene, two synthetic oligonucleotides (SEQ ID NO: 7 and SEQ ID NO: 8) for PCR amplification were synthesized. The plasmid pS947 (flaA-pBK-CMV) was used as a template for the PCR amplification. PCR amplification was performed and the 462 bp amplified fragment was ligated into the pCRII vector (Mead, D.A. et al. (1991) Bio/Technology 9: 657-663). The constructed plasmid was designated pS991. The sequence of the construct was confirmed by ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer).

The DNA encoding the middle part of the *flaA* gene was isolated by agarose gel electrophoresis as a 774 bp *Eco*RI / *Nhe*I fragment from the plasmid pS947. This fragment was ligated together with a 327 bp *Nhe*I / *Bgl*II fragment from pS989 and a 438 bp *Nde*I / *Eco*RI fragment from pS991 into a *Nde*I / *Bam*HI-digested pET3a (Studier, F.W. et al. (1990) Methods Enzymol. 185, 60-89). The generated plasmid was designated pS997.

#### (e) Construction of expression vector for recombinant *Helicobacter pylori* FlaB protein

In order to produce high levels of recombinant FlaB protein (SEQ ID NO: 4), the expression vector pS1000 was constructed. The vector contained the *Helicobacter pylori* *flaB* gene under control of the T7 promoter.

To generate convenient restriction sites for the 5'-end of the *flaB* gene, two synthetic oligonucleotides (SEQ ID NO: 9 and SEQ ID NO: 10) for PCR-amplification were synthesized. The plasmid pS948 (flaB-pBK-CMV) was used as a template for the PCR-amplification. PCR-amplification was performed and the 478 bp amplified fragment was ligated into the TA-vector (Mead, D.A. et al. (1991) Bio/Technology 9: 657-663). The

sequence of the construct was confirmed by PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing (PERKIN ELMER) according to manufacturers protocol. The constructed plasmid was designated pS998.

- 5 In order to change restriction sites in the 3'-end of the *flaB* gene, two synthetic oligonucleotides (SEQ ID NO: 11 and SEQ ID NO: 12) for PCR-amplification were synthesized. The plasmid pS948 (*flaB*-pBK-CMW) was used as a template for the PCR-amplification. PCR amplification was performed and the 1349 bp amplified fragment was ligated into the TA-vector (Mead, D.A. et al. (1991) Bio/Technology 9: 657-663). The  
10 constructed plasmid was designated pA. The sequence of the construct was confirmed by PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing (PERKIN ELMER). The amplified fragment was digested with *HindIII* and *NcoI* generating a 1158 bp fragment. This fragment was cloned into pRSETB and the constructed plasmid was designated pS999. The sequence of the construct was confirmed by PRISM Ready  
15 Reaction DyeDeoxy Terminator Cycle Sequencing (PERKIN ELMER).

- The DNA encoding the 5'-part of the *flaB* gene was isolated by gel electrophoresis as a 392 bp *NdeI*-*HindIII* fragment from the plasmid pS998 (template pS948 *flaB*-pBK-CMV). This fragment was ligated together with a 1230 bp *HindIII*-*BamHI* fragment from pS999  
20 and a 4.6 kB *NdeI*-*BamHI* fragment from T7 vector pS637 (pET-3a) (Studier, F.W. et al. (1990) Methods Enzymol. 185, 60-89). The resulting expression vector was designated pS1000.

(f) Host strains and bacterial cultures

- 25 The expression vector pS997 (*flaA*) or pS1000 (*flaB*) was transformed into the following *E. coli* host strains; BL21(DE3); BL21(DE3)pLysS; and BL21(DE3)pLysE. The expression experiments were carried out essentially as described by Studier et al. (*supra*). The bacteria were grown in LB (4) medium containing 50 µg/ml carbenicillin. In addition,  
30 when BL21(DE3)pLysS and BL21(DE3)pLysE were used, the medium was supplemented with 20 µg/ml chloramphenicol. For induction of the T7 expression system, the cultures

were grown to a density of approximately  $OD_{600} = 0.5$ , and then supplemented with 0.4 mM IPTG for induction. The cells were harvested about 180 minutes after induction. The host strain that gave the highest expression level for plasmid pS997 and plasmid pS1000 was BL21(DE3)pLysE and BL21 (DE3) pLysS respectively.

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(g) Purification of inclusion bodies of recombinant FlaA och FlaB produced in *E. coli*

Preparation of soluble *E. coli* proteins:

One liter fresh bacteria culture was centrifuged at  $11,300 \times g$  for 15 min at  $+4^{\circ}\text{C}$ . The  
10 resulting cell pellet was suspended in 10 ml of buffer (40 mM Tris-HCl, 0.1 mM EDTA, pH 8.2, 2 mM Pefablock SC (Boehringer Mannheim, Germany)). The suspension was transferred to JA-20 tubes (Beckman) and frozen at  $-20^{\circ}\text{C}$ . The pellet was thawed in water at room temperature and thereafter sonicated for  $10 \times 10 \text{ sec} \times 10 \text{ cycles}$  (Soniprep 150, MSE Scientific Instruments) in an ice-waterbath. The freeze-thaw and sonication procedure  
15 was repeated once. The suspension was centrifuged at  $23700 \times g$  for 15 min at  $+4^{\circ}\text{C}$ . The supernatant containing soluble proteins was collected. The pellet was resuspended in buffer as above and the whole freeze-thaw-sonication procedure was repeated once. The two supernatants was combined and filtered through a  $0.45 \mu\text{m}$  filter. The pellet was suspended in 10 ml of 40 mM Tris-HCl, 0.1 mM EDTA, pH 8.2 and frozen at  $-20^{\circ}\text{C}$ .

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Wash of inclusion bodies:

The pellet suspension containing either insoluble FlaA or FlaB was centrifuged at  $23300 \times g$  for 15 min at  $+4^{\circ}\text{C}$  and resuspended in 10 ml of wash buffer (100 mM Tris pH 7.0, 5 mM EDTA, 2 M urea, 2% Triton X-100. The suspension was centrifuged at  $23300 \times g$  for 30  
25 min at  $+4^{\circ}\text{C}$ . The pellet was resuspended in the wash buffer and the washing procedure was repeated twice. The final pellet was suspended in 100 mM Tris, 5 mM EDTA pH 7.0 and centrifuged as above. The resulting pellets were stored at  $-20^{\circ}\text{C}$ .

Denaturation/refolding experiments:

The washed pellets containing either insoluble FlaA or FlaB were resolved in denaturation buffer (50 mM glycine, 8 M GuHCl, pH 9.6) and centrifuged at 64,000 x g for 30 min at +4°C. The supernatant was filtered through a 0.2 µm filter and the protein concentration was determined by BCA-assay (Pierce, The Netherlands). Supernatant containing  
5 denatured protein could be stored at +4°C. The supernatant was diluted to 1–3 mg/ml with 50 mM glycine, 1 mM EDTA, 10 % sucrose, 4 M urea, pH 9.6, and dialyzed over night at +4°C, against the same buffer. The dialysis buffer was changed to 60 mM ethanolamine, 10 % sucrose, 1 mM EDTA pH 9.6 and dialysis continued over night at +4°C. The refolded sample was centrifuged at 10000 x g, for 5-10 minutes at +4°C. The supernatant contained  
10 the refolded protein. Usually 75% of the protein content was in the soluble fraction. The FlaA and FlaB protein was in solution if stored at +4°C but precipitated if stored at -20°C.

(h) Study of the antigenicity of recombinant FlaA and FlaB proteins

15 In order to produce antisera against recombinant FlaA or FlaB, these proteins were cut out from SDS-PAGE gels stained with Coomassie Brilliant Blue. About 500 µg of each was used for a total of four boosters immunisation. The resulting polyclonal antibodies against FlaA and FlaB were immunoreactive against respective antigen and were crossreactive against each other as determined by Western Immunoblotting and ELISA detection. Both  
20 polyclonal antisera were immunoreactive with purified flagellar preparations from *Helicobacter pylori* strains E32 or E50 as determined by Western Immunoblotting. Recombinant FlaA but not recombinant FlaB was immunoreactive with a monoclonal antibody (Mab104a) raised against purified *Helicobacter pylori* FlaA (Kostrzynska, M. et al. (1991) J. Bacteriol. 173(3) 937-946). Both recombinant FlaA and FlaB were  
25 immunoreactive with monoclonal antibodies raised against purified flagellar preparations from *Helicobacter pylori* strain E32.

## EXAMPLES

EXAMPLE 1: *Helicobacter pylori* flagellin

## 5 1.1. Infection and immunization

After a minimum of one week of acclimatisation, BALB/c mice were infected with a type 2 strain of *H. pylori* (strain 244, originally isolated from an ulcer patient). This strain had earlier proven to be a good colonizer of the mouse stomach. Bacteria from a stock kept at  
10 -70°C were grown overnight, in Brucella broth supplemented with 10% fetal calf serum, at +37°C in a microaerophilic atmosphere (10% CO<sub>2</sub>, 5% O<sub>2</sub>). The animals were given an oral dose of omeprazole (400 µmol/kg) in order to decrease acid secretion and improve subsequent survival of *Helicobacter pylori*. At 3 and 6 h. post omeprazole, animals were given an inoculation of approximately 10<sup>8</sup> fresh *H. pylori* strain 244. Infection was checked  
15 (see below) in control animals 2-3 weeks after the inoculation, prior to start of the experiment.

One month after infection, four groups of mice (10 animals/group) were immunized perorally 4 times over a 34 day period (days 1, 15, 25 and 35) as follows:

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- Group 1: Control, vehicle (PBS)
- Group 2: Cholera toxin (CT), 10 µg / animal
- Group 3: H.p. flagellin, 100 µg / animal + 10 µg CT
- Group 4: Membrane proteins, 500 µg / animal + 10 µg CT

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As a positive control, the mice in group 4 were immunized with crude membrane proteins from *H. pylori* strain 244. The animals in group 3 and 4 were also given 10 mg of cholera toxin with each immunization, as an adjuvant. A total volume of 0.3 ml was given at each immunization. Omeprazole (400 µmol/kg) was given orally to the animals 2-3 h prior to  
30 immunization in order to protect the antigens from acid degradation. The animals were sacrificed 4 weeks after the final immunization.

### 1.2. Analysis of infection

The mice were sacrificed by CO<sub>2</sub> and cervical dislocation. The abdomen was opened and the stomach removed. After cutting the stomach along the greater curvature, it was rinsed in saline. An area of 25 mm<sup>2</sup> of the mucosa from the antrum and corpus was scraped separately with a surgical scalpel. The mucosa scraping was suspended in Brucella broth and plated onto Blood Skirrow plates. The plates were incubated under microaerophilic conditions for 3-7 days and the CFU (colony-forming units) value was determined by counting the number of colonies. The identity of *H. pylori* was ascertained by urease and catalase test and by direct microscopy or Gram staining.

All control animals, as well as those receiving CT were infected in both antrum and corpus (Fig. 1). Animals actively immunized with H.p. flagellin, had significantly ( $p < 0.01$ ) decreased bacterial content (CFU values) compared to controls. In the flagellin group, no bacteria could be detected in the corpus of five mice and one was negative also in the antrum. A smaller but significant ( $p < 0.01$ ) decrease in CFU was also observed in the antrum following vaccination with *H. pylori* membrane proteins.

### 1.3. Analysis of immune response

Serum antibodies were collected from blood drawn by heart-puncture in conjunction with termination of the study. Prior to centrifugation, the blood was diluted with equal amount of PBS. The serum was kept at -20°C until analysis. Serum antibodies were measured using an ELISA wherein purified H.p. flagellin was plated followed by addition of serum in a dilution series. An alkaline phosphatase-labelled goat anti-mouse Ig antibody was used as conjugate. Results (Fig. 2) were read as titers from plotting OD readouts and comparing to a standard curve. Uninfected controls had values below 80. In *H. pylori* infected control mice the antibody titers were increased to 189. In infected animals given flagellin immunization these levels were increased to 424.

#### 1.4. Passive protection

The objective of this study was to investigate whether binding to *H. pylori* of monoclonal antibodies, directed to *H. pylori* flagellin, could decrease or prevent colonisation of the bacteria in mice.

Three groups of mice (10 animals/group) were used. One group was challenged with a mixture of freshly grown *H. pylori*, strain 244, and a monoclonal antibody, HP50F-48:13;1. The mixture was incubated 10 min at room temperature before inoculation to the animals. For comparison, one group was inoculated with *H. pylori* strain 244 only, and one group was given a mixture of *H. pylori* strain 244 and a control monoclonal antibody, directed against the *E. coli* heat stable protein (ST). All inoculations were done perorally and at a volume of 0.3 ml.

Two weeks after challenge the mice were sacrificed and analyzed for presence of gastric *H. pylori* as described above (Fig. 3). All control animals, both those who received bacteria only as well as those who received bacteria and the *E. coli* ST MAb, were well infected. In contrast, none of the animals inoculated with the mixture of bacteria and flagellin MAb were infected, a statistically significant difference ( $p < 0.001$ ).

#### EXAMPLE 2: Recombinant FlaA (rFlaA)

The experiment was performed as in Example 1, with the exception that the animals were sacrificed and evaluated 10 days after the last immunization (day 45). Three groups of animals (10/group) was treated according to the scheme below:

- Group 1: Control, vehicle (PBS)
- Group 2: Cholera toxin (CT), 10 µg/animal

Group 3: rFlaA, 100 µg/animal + 10 µg CT

The response to oral immunization was evaluated by H.p. CFU in the gastric antrum and corpus mucosa. In stomach and duodenum, serum IgG antibodies, as well as mucosal Ig  
5 and IgA antibodies were determined.

Mucosal antibodies were collected by the following technique. One half of the rinsed stomach was placed mucosal side up on a piece of paper. Likewise the duodenum was cut open and placed mucosal side up. One standardised round filter paper (30.4 mm<sup>2</sup>) was  
10 placed on the antrum and one on the corpus musosa. After 10 minutes both papers were transferred to one tube with 200 µl special buffer containing protease inhibitors. A paper strip, 4.8x19 mm (91.2 mm<sup>2</sup>) was in the same way placed on the duodenum mucosa and was subsequently placed in a separate tube with buffer. After a minimum of one hour  
15 extraction of the filter papers, the buffer solutions from the 10 mice within each group was pooled. The pooled solutions were either used directly for ELISA measurements of antibody concentration or kept frozen at -20°C.

Serum antibodies were collected from blood drawn by heart-puncture in conjunction with termination of the study. Prior to centrifugation, the blood was diluted with equal amount  
20 of PBS. The serum was kept at -20°C until analysis.

Mucosal antibodies were measured using an ELISA wherein plates were coated with rFlaA followed by addition of mucosal extract. The ELISA was developed with alkaline phosphatase-labelled anti-mouse-Ig or anti-mouse-IgA antibodies. The anti-Ig antibodies  
25 were of an anti-heavy/anti-light chain type, which will normally detect all types of antibodies. Standard curves were created by coating known amounts of mouse IgA and Ig.

Serum Ig antibodies were measured using an ELISA wherein plates were coated either with a particulate fraction (membrane protein; m.p.) of *H. pylori* strain 244 or with rFlaA followed by addition of different dilutions of serum. The ELISA was developed with alkaline phosphatase-labelled anti-mouse-Ig-antibodies as described above.

5

### Results

All control animals and CT treated animals were well infected in both antrum and corpus. In animals receiving rFlaA + CT the degree of colonization was significantly lower in  
10 corpus mucosa (\* $p < 0.05$ ) (Fig. 4). In the rFlaA + CT group, one animal had no *H. pylori* in antrum and 3 animals had no *H. pylori* in corpus.

Systemic immune response measured as IgG in serum showed immune reactivity to the infected strain 244 (control and CT groups). Only in animals receiving rFlaA + CT could  
15 an immune response towards FlaA be recorded (Fig 5).

Local (mucosal) immune response measured as IgA showed specific immune reactivity against FlaA after immunization with FlaA + CT. No such response was seen in control animals, see Fig. 6.

20

It can be concluded that recombinant FlaA can induce an eradicated immune response capable of decreasing or clearing an *H. pylori* infection.

### EXAMPLE 3: Recombinant FlaB (rFlaB)

The experiment was performed and analyzed as described in Example 2. Three groups of animals (10/group) was treated according to the scheme below:

25 Group 1: Control, vehicle (PBS)

Group 2: Cholera toxin (CT), 10 µg/animal

Group 3: rFlaB, 100 µg/animal + 10 µg CT

### Results

5

All control animals and CT treated animals were well infected in both antrum and corpus. In animals receiving rFlaB + CT the degree of colonization (cfu) was significantly lower in antrum mucosa, geometric mean 1005 vs 83 (\*p<0.05, Wilcoxon-Mann-Whittney Sign Rank Test). In the rFlaB + CT group, 3/10 animals were free of *H. pylori* in the antrum.

10 Only in animals receiving rFlaB could a serum IgG response to FlaB be measured i.e.  $85.7 \pm 46.0$  µg/ml (mean  $\pm$  SEM, n=10).

Local mucosal response to oral immunization was measured as specific IgA antibodies to rFlaB in stomach and duodenal mucosa. The values were  $3.3 \pm 2.0$  ng/ml and  $12.1 \pm 6.6$  ng/ml (mean $\pm$ SEM, n=10) in stomach and duodenal mucosa respectively.

It can be concluded that recombinant FlaB can induce an eradicated immune response capable of decreasing or clearing an *H. pylori* infection.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: Astra Aktiebolag
- (B) STREET: Västra Mälarehamnen 9
- (C) CITY: Södertälje
- (E) COUNTRY: Sweden
- (F) POSTAL CODE (ZIP): S-151 85
- (G) TELEPHONE: +46 8 553 260 00
- (H) TELEFAX: +46 8 553 288 20

(ii) TITLE OF INVENTION: BACTERIAL ANTIGENS AND VACCINE COMPOSITIONS

(iii) NUMBER OF SEQUENCES: 12

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1800 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 139..1671
- (D) OTHER INFORMATION: /product= "FlaA protein"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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|-------------------------------------------------------------------|-----|
| TTTATTATAG CCCATTTTCA TGCTCTTTTA AATTTTGCTT TTAAAGTAAA GCCCTTTAAA | 60  |
| ATTTCAAAC TTAACCGATA ATAGTTCCAA CCAAAGCAA GGATGCCTTT GGGTTTTTTA   | 120 |
| TAACAAGGAG TTACAACA ATG GCT TTT CAG GTC AAT ACA AAT ATC AAT GCG   | 171 |
| Met Ala Phe Gln Val Asn Thr Asn Ile Asn Ala                       |     |
| 1 5 10                                                            |     |

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| ATG AAT GCG CAT GTG CAA TCC GCA CTC ACT CAA AAT GCG CTT AAA ACT | 219 |
| Met Asn Ala His Val Gln Ser Ala Leu Thr Gln Asn Ala Leu Lys Thr |     |
| 15 20 25                                                        |     |
| TCA TTG GAG AGA TTG AGT TCA GGT TTA AGG ATT AAT AAA GCG GCT GAT | 267 |
| Ser Leu Glu Arg Leu Ser Ser Gly Leu Arg Ile Asn Lys Ala Ala Asp |     |
| 30 35 40                                                        |     |
| GAC GCA TCA GGC ATG ACG GTG GCG GAT TCT TTG CGT TCA CAA GCG AGC | 315 |
| Asp Ala Ser Gly Met Thr Val Ala Asp Ser Leu Arg Ser Gln Ala Ser |     |
| 45 50 55                                                        |     |
| AGT TTG GGT CAA GCG ATT GCC AAC ACG AAT GAC GGC ATG GGG ATT ATC | 363 |
| Ser Leu Gly Gln Ala Ile Ala Asn Thr Asn Asp Gly Met Gly Ile Ile |     |
| 60 65 70 75                                                     |     |
| CAG GTT GCG GAT AAG GCT ATG GAT GAG CAG TTG AAA ATC TTA GAC ACC | 411 |
| Gln Val Ala Asp Lys Ala Met Asp Glu Gln Leu Lys Ile Leu Asp Thr |     |
| 80 85 90                                                        |     |
| GTT AAG GTT AAA GCG ACT CAA GCG GCT CAA GAC GGG CAA ACT ACA GAA | 459 |
| Val Lys Val Lys Ala Thr Gln Ala Ala Gln Asp Gly Gln Thr Thr Glu |     |
| 95 100 105                                                      |     |
| TCT CGT AAA GCG ATT CAA TCT GAC ATC GTT CGT TTG ATT CAA GGT TTA | 507 |
| Ser Arg Lys Ala Ile Gln Ser Asp Ile Val Arg Leu Ile Gln Gly Leu |     |
| 110 115 120                                                     |     |
| GAC AAT ATC GGT AAC ACG ACT ACT TAT AAC GGG CAA GCG TTA TTG TCT | 555 |
| Asp Asn Ile Gly Asn Thr Thr Thr Tyr Asn Gly Gln Ala Leu Leu Ser |     |
| 125 130 135                                                     |     |
| GGT CAA TTC ACT AAC AAA GAA TTC CAA GTA GGG GCT TAT TCT AAC CAA | 603 |
| Gly Gln Phe Thr Asn Lys Glu Phe Gln Val Gly Ala Tyr Ser Asn Gln |     |
| 140 145 150 155                                                 |     |
| AGT ATT AAG GCT TCT ATC GGC TCT ACC ACT TCC GAT AAA ATC GGT CAG | 651 |
| Ser Ile Lys Ala Ser Ile Gly Ser Thr Thr Ser Asp Lys Ile Gly Gln |     |
| 160 165 170                                                     |     |
| GTT CGT ATC GCT ACA GGC GCG TTA ATC ACG GCT TCT GGG GAT ATT AGC | 699 |
| Val Arg Ile Ala Thr Gly Ala Leu Ile Thr Ala Ser Gly Asp Ile Ser |     |
| 175 180 185                                                     |     |
| TTG ACT TTT AAA CAA GTG GAT GGC GTG AAT GAT GTA ACT TTA GAG AGC | 747 |
| Leu Thr Phe Lys Gln Val Asp Gly Val Asn Asp Val Thr Leu Glu Ser |     |
| 190 195 200                                                     |     |
| GTG AAA GTC TCT AGT TCA GCA GGC ACA GGG ATT GGC GTG TTA GCG GAA | 795 |
| Val Lys Val Ser Ser Ser Ala Gly Thr Gly Ile Gly Val Leu Ala Glu |     |
| 205 210 215                                                     |     |
| GTG ATT AAC AAA AAC TCT AAC CGA ACA GGG GTT AAA GCT TAT GCG AGC | 843 |
| Val Ile Asn Lys Asn Ser Asn Arg Thr Gly Val Lys Ala Tyr Ala Ser |     |
| 220 225 230 235                                                 |     |

|                                                                 |      |
|-----------------------------------------------------------------|------|
| GTT ATC ACC ACG AGC GAT GTG GCG GTC CAG TCA GGA AGT TTG AGT AAT | 891  |
| Val Ile Thr Thr Ser Asp Val Ala Val Gln Ser Gly Ser Leu Ser Asn |      |
| 240 245 250                                                     |      |
| TTA ACC TTA AAT GGG ATC CAT TTG GGT AAT ATC GCA GAT ATT AAG AAA | 939  |
| Leu Thr Leu Asn Gly Ile His Leu Gly Asn Ile Ala Asp Ile Lys Lys |      |
| 255 260 265                                                     |      |
| AAT GAC TCA GAC GGA CGA TTG GTT GCA GCG ATC AAT GCG GTT ACT TCA | 987  |
| Asn Asp Ser Asp Gly Arg Leu Val Ala Ala Ile Asn Ala Val Thr Ser |      |
| 270 275 280                                                     |      |
| GAA ACC GGT GTG GAA GCT TAT ACG GAT CAA AAA GGG CGC TTG AAT TTG | 1035 |
| Glu Thr Gly Val Glu Ala Tyr Thr Asp Gln Lys Gly Arg Leu Asn Leu |      |
| 285 290 295                                                     |      |
| CGC AGT ATA GAT GGT CGT GGG ATT GAA ATT AAA ACC GAT AGC GTC AGT | 1083 |
| Arg Ser Ile Asp Gly Arg Gly Ile Glu Ile Lys Thr Asp Ser Val Ser |      |
| 300 305 310 315                                                 |      |
| AAC GGG CCT AGT GCT TTA ACG ATG GTC AAT GGC GGT CAG GAT TTA ACA | 1131 |
| Asn Gly Pro Ser Ala Leu Thr Met Val Asn Gly Gly Gln Asp Leu Thr |      |
| 320 325 330                                                     |      |
| AAA GGC TCT ACT AAC TAT GGG AGG CTT TCT CTC ACA CGA TTA GAC GCT | 1179 |
| Lys Gly Ser Thr Asn Tyr Gly Arg Leu Ser Leu Thr Arg Leu Asp Ala |      |
| 335 340 345                                                     |      |
| AAA AGC ATC AAT GTC GTT TCG GCT TCT GAC TCA CAG CAT TTA GGC TTC | 1227 |
| Lys Ser Ile Asn Val Val Ser Ala Ser Asp Ser Gln His Leu Gly Phe |      |
| 350 355 360                                                     |      |
| ACA GCG ATT GGT TTT GGG GAA TCT CAA GTG GCA GAA ACC ACG GTG AAT | 1275 |
| Thr Ala Ile Gly Phe Gly Glu Ser Gln Val Ala Glu Thr Thr Val Asn |      |
| 365 370 375                                                     |      |
| TTG CGC GAT GTT ACT GGG AAT TTT AAC GCT AAT GTC AAA TCA GCT AGT | 1323 |
| Leu Arg Asp Val Thr Gly Asn Phe Asn Ala Asn Val Lys Ser Ala Ser |      |
| 380 385 390 395                                                 |      |
| GGT GCG AAC TAT AAC GCC GTC ATT GCT AGC GGT AAT CAG AGC TTG GGA | 1371 |
| Gly Ala Asn Tyr Asn Ala Val Ile Ala Ser Gly Asn Gln Ser Leu Gly |      |
| 400 405 410                                                     |      |
| TCT GGG GTT ACA ACC TTG AGA GGC GCG ATG GTG GTG ATT GAT ATT GCC | 1419 |
| Ser Gly Val Thr Thr Leu Arg Gly Ala Met Val Val Ile Asp Ile Ala |      |
| 415 420 425                                                     |      |
| GAG TCT GCG ATG AAA ATG TTG GAT AAA GTC CGC TCT GAT TTA GGT TCT | 1467 |
| Glu Ser Ala Met Lys Met Leu Asp Lys Val Arg Ser Asp Leu Gly Ser |      |
| 430 435 440                                                     |      |

GTG CAA AAT CAA ATG ATT AGC ACC GTG AAT AAC ATC AGC ATC ACT CAA 1515  
 Val Gln Asn Gln Met Ile Ser Thr Val Asn Asn Ile Ser Ile Thr Gln  
 445 450 455  
 GTG AAT GTT AAA GCG GCT GAA TCT CAA ATC AGG GAT GTG GAT TTC GCT 1563  
 Val Asn Val Lys Ala Ala Glu Ser Gln Ile Arg Asp Val Asp Phe Ala  
 460 465 470 475  
 GAA GAG AGT GCG AAT TTC AAT AAA AAC AAC ATT TTG GCG CAA TCA GGC 1611  
 Glu Glu Ser Ala Asn Phe Asn Lys Asn Asn Ile Leu Ala Gln Ser Gly  
 480 485 490  
 AGC TAT GCG ATG AGT CAA GCC AAT ACC GTC CAA CAA AAT ATC TTA AGG 1659  
 Ser Tyr Ala Met Ser Gln Ala Asn Thr Val Gln Gln Asn Ile Leu Arg  
 495 500 505  
 CTT TTA ACT TAG TTTAAGAAA GGTGTTTGTA TGGGGCTAAC GCTTTAAGCG 1711  
 Leu Leu Thr \*  
 510  
 TTGGCTTTTC GCTTTAATTT TTAATTCTTT TTTAATAAAA TACTCTTTT GATTCTTTT 1771  
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## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 511 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Phe Gln Val Asn Thr Asn Ile Asn Ala Met Asn Ala His Val  
 1 5 10 15  
 Gln Ser Ala Leu Thr Gln Asn Ala Leu Lys Thr Ser Leu Glu Arg Leu  
 20 25 30  
 Ser Ser Gly Leu Arg Ile Asn Lys Ala Ala Asp Asp Ala Ser Gly Met  
 35 40 45  
 Thr Val Ala Asp Ser Leu Arg Ser Gln Ala Ser Ser Leu Gly Gln Ala  
 50 55 60  
 Ile Ala Asn Thr Asn Asp Gly Met Gly Ile Ile Gln Val Ala Asp Lys  
 65 70 75 80  
 Ala Met Asp Glu Gln Leu Lys Ile Leu Asp Thr Val Lys Val Lys Ala  
 85 90 95  
 Thr Gln Ala Ala Gln Asp Gly Gln Thr Thr Glu Ser Arg Lys Ala Ile  
 100 105 110

Gln Ser Asp Ile Val Arg Leu Ile Gln Gly Leu Asp Asn Ile Gly Asn  
 115 120 125  
 Thr Thr Thr Tyr Asn Gly Gln Ala Leu Leu Ser Gly Gln Phe Thr Asn  
 130 135 140  
 Lys Glu Phe Gln Val Gly Ala Tyr Ser Asn Gln Ser Ile Lys Ala Ser  
 145 150 155 160  
 Ile Gly Ser Thr Thr Ser Asp Lys Ile Gly Gln Val Arg Ile Ala Thr  
 165 170 175  
 Gly Ala Leu Ile Thr Ala Ser Gly Asp Ile Ser Leu Thr Phe Lys Gln  
 180 185 190  
 Val Asp Gly Val Asn Asp Val Thr Leu Glu Ser Val Lys Val Ser Ser  
 195 200 205  
 Ser Ala Gly Thr Gly Ile Gly Val Leu Ala Glu Val Ile Asn Lys Asn  
 210 215 220  
 Ser Asn Arg Thr Gly Val Lys Ala Tyr Ala Ser Val Ile Thr Thr Ser  
 225 230 235 240  
 Asp Val Ala Val Gln Ser Gly Ser Leu Ser Asn Leu Thr Leu Asn Gly  
 245 250 255  
 Ile His Leu Gly Asn Ile Ala Asp Ile Lys Lys Asn Asp Ser Asp Gly  
 260 265 270  
 Arg Leu Val Ala Ala Ile Asn Ala Val Thr Ser Glu Thr Gly Val Glu  
 275 280 285  
 Ala Tyr Thr Asp Gln Lys Gly Arg Leu Asn Leu Arg Ser Ile Asp Gly  
 290 295 300  
 Arg Gly Ile Glu Ile Lys Thr Asp Ser Val Ser Asn Gly Pro Ser Ala  
 305 310 315 320  
 Leu Thr Met Val Asn Gly Gly Gln Asp Leu Thr Lys Gly Ser Thr Asn  
 325 330 335  
 Tyr Gly Arg Leu Ser Leu Thr Arg Leu Asp Ala Lys Ser Ile Asn Val  
 340 345 350  
 Val Ser Ala Ser Asp Ser Gln His Leu Gly Phe Thr Ala Ile Gly Phe  
 355 360 365  
 Gly Glu Ser Gln Val Ala Glu Thr Thr Val Asn Leu Arg Asp Val Thr  
 370 375 380  
 Gly Asn Phe Asn Ala Asn Val Lys Ser Ala Ser Gly Ala Asn Tyr Asn  
 385 390 395 400

Ala Val Ile Ala Ser Gly Asn Gln Ser Leu Gly Ser Gly Val Thr Thr  
 405 410 415

Leu Arg Gly Ala Met Val Val Ile Asp Ile Ala Glu Ser Ala Met Lys  
 420 425 430

Met Leu Asp Lys Val Arg Ser Asp Leu Gly Ser Val Gln Asn Gln Met  
 435 440 445

Ile Ser Thr Val Asn Asn Ile Ser Ile Thr Gln Val Asn Val Lys Ala  
 450 455 460

Ala Glu Ser Gln Ile Arg Asp Val Asp Phe Ala Glu Glu Ser Ala Asn  
 465 470 475 480

Phe Asn Lys Asn Asn Ile Leu Ala Gln Ser Gly Ser Tyr Ala Met Ser  
 485 490 495

Gln Ala Asn Thr Val Gln Gln Asn Ile Leu Arg Leu Leu Thr \*

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1800 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 138..1682
- (D) OTHER INFORMATION: /product= "FlaB protein"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TATTAATGAA TGATTGTAGC ATAGAATTTT GACTAAACGA TTCATTAAAC CATAAAAACC 60

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ATGCAAAGGA TGCAAAC ATG AGT TTT AGG ATA AAT ACC AAT ATC GCC GCT 170  
 Met Ser Phe Arg Ile Asn Thr Asn Ile Ala Ala  
 515 520

TTA ACT TCT CAT GCG GTA GGG GTT CAA AAC AAC AGA GAC CTT TCA AGT 218  
 Leu Thr Ser His Ala Val Gly Val Gln Asn Asn Arg Asp Leu Ser Ser  
 525 530 535

TCG CTT GAA AAG TTA AGC TCA GGG CTT AGG ATC AAT AAA GCC GCT GAC 266  
 Ser Leu Glu Lys Leu Ser Ser Gly Leu Arg Ile Asn Lys Ala Ala Asp  
 540 545 550

|                                                                                                                                                       |     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| GAT TCT AGT GGG ATG GCG ATC GCT GAT AGC TTA AGG AGT CAA AGC GCG<br>Asp Ser Ser Gly Met Ala Ile Ala Asp Ser Leu Arg Ser Gln Ser Ala<br>555 560 565 570 | 314 |
| AAT TTA GGC CAG GCG ATT CGC AAT GCT AAT GAC GCT ATT GGT ATG GTT<br>Asn Leu Gly Gln Ala Ile Arg Asn Ala Asn Asp Ala Ile Gly Met Val<br>575 580 585     | 362 |
| CAA ACC GCA GAT AAA GCG ATG GAT GAG CAA ATC AAA ATC TTA GAC ACC<br>Gln Thr Ala Asp Lys Ala Met Asp Glu Gln Ile Lys Ile Leu Asp Thr<br>590 595 600     | 410 |
| ATT AAA ACC AAA GCC GTT CAA GCC GCT CAA GAT GGG CAA ACT TTA GAA<br>Ile Lys Thr Lys Ala Val Gln Ala Ala Gln Asp Gly Gln Thr Leu Glu<br>605 610 615     | 458 |
| AGC CGA AGA GCG CTC CAA AGC GAT ATT CAA AGG TTG TTA GAA GAA CTA<br>Ser Arg Arg Ala Leu Gln Ser Asp Ile Gln Arg Leu Leu Glu Glu Leu<br>620 625 630     | 506 |
| GAC AAT ATC GCT AAC ACC ACA AGC TTT AAC GGC CAA CAA ATG CTT TCA<br>Asp Asn Ile Ala Asn Thr Thr Ser Phe Asn Gly Gln Gln Met Leu Ser<br>635 640 645 650 | 554 |
| GGA AGT TTT TCT AAC AAA GAA TTT CAA ATT GGC GCG TAT TCT AAC ACC<br>Gly Ser Phe Ser Asn Lys Glu Phe Gln Ile Gly Ala Tyr Ser Asn Thr<br>655 660 665     | 602 |
| ACG GTT AAA GCG TCT ATT GGC TCA ACA AGC TCA GAT AAG ATT GGG CAT<br>Thr Val Lys Ala Ser Ile Gly Ser Thr Ser Ser Asp Lys Ile Gly His<br>670 675 680     | 650 |
| GTA CGC ATG GAA ACT TCT TCT TTT AGC GGT GCA GGC ATG CTC GCT AGC<br>Val Arg Met Glu Thr Ser Ser Phe Ser Gly Ala Gly Met Leu Ala Ser<br>685 690 695     | 698 |
| GCG GCG GCG CAA AAC TTG ACT GAA GTG GGA TTG AAT TTC AAA CAA GTC<br>Ala Ala Ala Gln Asn Leu Thr Glu Val Gly Leu Asn Phe Lys Gln Val<br>700 705 710     | 746 |
| AAT GGC GTG AAT GAT TAT AAG ATT GAA ACC GTG CGT ATT TCT ACA AGC<br>Asn Gly Val Asn Asp Tyr Lys Ile Glu Thr Val Arg Ile Ser Thr Ser<br>715 720 725 730 | 794 |
| GCT GGC ACT GGG ATT GGG GCG TTA AGC GAG ATT ATC AAT CGC TTT TCT<br>Ala Gly Thr Gly Ile Gly Ala Leu Ser Glu Ile Ile Asn Arg Phe Ser<br>735 740 745     | 842 |
| AAC ACC TTA GGC GTT AGG GCG TCT TAT AAT GTC ATG GCT ACC GGC GGC<br>Asn Thr Leu Gly Val Arg Ala Ser Tyr Asn Val Met Ala Thr Gly Gly<br>750 755 760     | 890 |

|                                                                 |      |
|-----------------------------------------------------------------|------|
| ACT CCC GTG CAA TCA GGA ACT GTG AGA GAA CTC ACC ATT AAT GGC GTA | 938  |
| Thr Pro Val Gln Ser Gly Thr Val Arg Glu Leu Thr Ile Asn Gly Val |      |
| 765 770 775                                                     |      |
| GAA ATT GGG ACC GTG AAT GAT GTG CAT AAA AAC GAC GCT GAT GGG AGA | 986  |
| Glu Ile Gly Thr Val Asn Asp Val His Lys Asn Asp Ala Asp Gly Arg |      |
| 780 785 790                                                     |      |
| TTG ACT AAC GCG ATC AAC TCC GTC AAA GAC AGG ACC GGC GTA GAA GCG | 1034 |
| Leu Thr Asn Ala Ile Asn Ser Val Lys Asp Arg Thr Gly Val Glu Ala |      |
| 795 800 805 810                                                 |      |
| AGC TTG GAT ATT CAA GGG CGC ATT AAT TTG CAC TCC ATT GAC GGG CGT | 1082 |
| Ser Leu Asp Ile Gln Gly Arg Ile Asn Leu His Ser Ile Asp Gly Arg |      |
| 815 820 825                                                     |      |
| GCG ATT TCT GTG CAT GCA GCG AGC GCG AGC GGT CAG GTT TTT GGG GGA | 1130 |
| Ala Ile Ser Val His Ala Ala Ser Ala Ser Gly Gln Val Phe Gly Gly |      |
| 830 835 840                                                     |      |
| GGG AAT TTT GCA GGG ATT TCT GGG ACA CAG CAT GCG GTG ATT GGG CGC | 1178 |
| Gly Asn Phe Ala Gly Ile Ser Gly Thr Gln His Ala Val Ile Gly Arg |      |
| 845 850 855                                                     |      |
| TTA ACC TTA ACT AGA ACC GAC GCT AGA GAC ATC ATT GTA AGC GGT GTG | 1226 |
| Leu Thr Leu Thr Arg Thr Asp Ala Arg Asp Ile Ile Val Ser Gly Val |      |
| 860 865 870                                                     |      |
| AAT TTT AGC CAT GTG GGC TTT CAT TCC GCT CAA GGG GTG GCA GAA TAC | 1274 |
| Asn Phe Ser His Val Gly Phe His Ser Ala Gln Gly Val Ala Glu Tyr |      |
| 875 880 885 890                                                 |      |
| ACC GTG AAT TTG AGA GCG GTT AGG GGC ATT TTT GAT GCG AAT GTG GCT | 1322 |
| Thr Val Asn Leu Arg Ala Val Arg Gly Ile Phe Asp Ala Asn Val Ala |      |
| 895 900 905                                                     |      |
| TCA GCA GCC GGA GCG AAC GCT AAT GGC GCA CAA GCG GAG ACC AAT TCT | 1370 |
| Ser Ala Ala Gly Ala Asn Ala Asn Gly Ala Gln Ala Glu Thr Asn Ser |      |
| 910 915 920                                                     |      |
| CAA GGT ATA GGG GCT GGG GTA ACA AGC CTT AAG GGG GCG ATG ATT GTG | 1418 |
| Gln Gly Ile Gly Ala Gly Val Thr Ser Leu Lys Gly Ala Met Ile Val |      |
| 925 930 935                                                     |      |
| ATG GAT ATG GCA GAT TCT GCA CGC ACG CAA TTA GAC AAG ATC CGC TCG | 1466 |
| Met Asp Met Ala Asp Ser Ala Arg Thr Gln Leu Asp Lys Ile Arg Ser |      |
| 940 945 950                                                     |      |
| GAT ATG GGT TCG GTG CAA ATG GAA TTG GTT ACA ACC ATT AAT AAT ATT | 1514 |
| Asp Met Gly Ser Val Gln Met Glu Leu Val Thr Thr Ile Asn Asn Ile |      |
| 955 960 965 970                                                 |      |
| TCT GTA ACC CAA GTG AAT GTT AAA GCG GCT GAA TCT CAA ATC AGA GAC | 1562 |
| Ser Val Thr Gln Val Asn Val Lys Ala Ala Glu Ser Gln Ile Arg Asp |      |
| 975 980 985                                                     |      |

```

GTG GAT TTT GCT GAA GAG AGC GCG AAC TTT TCT AAA TAC AAT ATT TTG      1610
Val Asp Phe Ala Glu Glu Ser Ala Asn Phe Ser Lys Tyr Asn Ile Leu
          990                      995                      1000

GCG CAA AGC GGG AGT TTT GCT ATG GCG CAA GCG AAT GCG GTG CAA CAG      1658
Ala Gln Ser Gly Ser Phe Ala Met Ala Gln Ala Asn Ala Val Gln Gln
          1005                      1010                      1015

AAT GTC TTA AGG CTT TTA CAA TAA CAGCCCTTTT AATTCAAAAG GCGGTTAGCC      1712
Asn Val Leu Arg Leu Leu Gln *
          1020                      1025

CTTTTATCA GTTATTTTTA TAAGTTAGAA TGATGGATAT TTATCAAAAA AACTTACAAG      1772

CTCTTTTCAA AAAAGACCCT CTTTGTGTT      1800

```

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 515 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```

Met Ser Phe Arg Ile Asn Thr Asn Ile Ala Ala Leu Thr Ser His Ala
 1              5              10              15

Val Gly Val Gln Asn Asn Arg Asp Leu Ser Ser Ser Leu Glu Lys Leu
          20              25              30

Ser Ser Gly Leu Arg Ile Asn Lys Ala Ala Asp Asp Ser Ser Gly Met
          35              40              45

Ala Ile Ala Asp Ser Leu Arg Ser Gln Ser Ala Asn Leu Gly Gln Ala
          50              55              60

Ile Arg Asn Ala Asn Asp Ala Ile Gly Met Val Gln Thr Ala Asp Lys
          65              70              75              80

Ala Met Asp Glu Gln Ile Lys Ile Leu Asp Thr Ile Lys Thr Lys Ala
          85              90              95

Val Gln Ala Ala Gln Asp Gly Gln Thr Leu Glu Ser Arg Arg Ala Leu
          100             105             110

Gln Ser Asp Ile Gln Arg Leu Leu Glu Glu Leu Asp Asn Ile Ala Asn
          115             120             125

Thr Thr Ser Phe Asn Gly Gln Gln Met Leu Ser Gly Ser Phe Ser Asn
          130             135             140

```

Lys Glu Phe Gln Ile Gly Ala Tyr Ser Asn Thr Thr Val Lys Ala Ser  
 145 150 155 160  
 Ile Gly Ser Thr Ser Ser Asp Lys Ile Gly His Val Arg Met Glu Thr  
 165 170 175  
 Ser Ser Phe Ser Gly Ala Gly Met Leu Ala Ser Ala Ala Ala Gln Asn  
 180 185 190  
 Leu Thr Glu Val Gly Leu Asn Phe Lys Gln Val Asn Gly Val Asn Asp  
 195 200 205  
 Tyr Lys Ile Glu Thr Val Arg Ile Ser Thr Ser Ala Gly Thr Gly Ile  
 210 215 220  
 Gly Ala Leu Ser Glu Ile Ile Asn Arg Phe Ser Asn Thr Leu Gly Val  
 225 230 235 240  
 Arg Ala Ser Tyr Asn Val Met Ala Thr Gly Gly Thr Pro Val Gln Ser  
 245 250 255  
 Gly Thr Val Arg Glu Leu Thr Ile Asn Gly Val Glu Ile Gly Thr Val  
 260 265 270  
 Asn Asp Val His Lys Asn Asp Ala Asp Gly Arg Leu Thr Asn Ala Ile  
 275 280 285  
 Asn Ser Val Lys Asp Arg Thr Gly Val Glu Ala Ser Leu Asp Ile Gln  
 290 295 300  
 Gly Arg Ile Asn Leu His Ser Ile Asp Gly Arg Ala Ile Ser Val His  
 305 310 315 320  
 Ala Ala Ser Ala Ser Gly Gln Val Phe Gly Gly Gly Asn Phe Ala Gly  
 325 330 335  
 Ile Ser Gly Thr Gln His Ala Val Ile Gly Arg Leu Thr Leu Thr Arg  
 340 345 350  
 Thr Asp Ala Arg Asp Ile Ile Val Ser Gly Val Asn Phe Ser His Val  
 355 360 365  
 Gly Phe His Ser Ala Gln Gly Val Ala Glu Tyr Thr Val Asn Leu Arg  
 370 375 380  
 Ala Val Arg Gly Ile Phe Asp Ala Asn Val Ala Ser Ala Ala Gly Ala  
 385 390 395 400  
 Asn Ala Asn Gly Ala Gln Ala Glu Thr Asn Ser Gln Gly Ile Gly Ala  
 405 410 415  
 Gly Val Thr Ser Leu Lys Gly Ala Met Ile Val Met Asp Met Ala Asp  
 420 425 430

Ser Ala Arg Thr Gln Leu Asp Lys Ile Arg Ser Asp Met Gly Ser Val  
 435 440 445

Gln Met Glu Leu Val Thr Thr Ile Asn Asn Ile Ser Val Thr Gln Val  
 450 455 460

Asn Val Lys Ala Ala Glu Ser Gln Ile Arg Asp Val Asp Phe Ala Glu  
 465 470 475 480

Glu Ser Ala Asn Phe Ser Lys Tyr Asn Ile Leu Ala Gln Ser Gly Ser  
 485 490 495

Phe Ala Met Ala Gln Ala Asn Ala Val Gln Gln Asn Val Leu Arg Leu  
 500 505 510

Leu Gln \*  
 515

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "PCR primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACACCCGGGG CTAGCGGTAA TCAGAGCTTG

30

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "PCR primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACACACTGCA GAGATCTTTA CTAAGTTAAA AGCCTTAAGA TATTT

45

## (2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "PCR primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ACAGTCGACC ATATGGCTTT TCAGGTCAAT ACAAAT

36

## (2) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "PCR primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ACACCCGGGG AATTCTTTGT TAGTGAATTG ACC

33

## (2) INFORMATION FOR SEQ ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "PCR primer"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGGAATTCAT ATGAGTTTGA GGATAAATAC CAATA

35

## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "PCR primer"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CGTGGTGTTA GAATACGCGC C

21

- (2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "PCR primer"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTATTGGTAT GGTCAAACC GC

22

- (2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 39 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "PCR primer"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CACACACCAT GGCTATTATT GTAAAAGCCT TAAGACATT

39



## CLAIMS

1. A polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide, or a modified form of the said polypeptide retaining functionally equivalent antigenicity,  
5 for use in inducing a protective immune response to *Helicobacter pylori* infection.
2. The polypeptide according to claim 1 which comprises the *Helicobacter pylori* polypeptide FlaA, or a modified form of the said polypeptide retaining functionally equivalent antigenicity, for use in inducing a protective immune response to  
10 *Helicobacter pylori* infection.
3. The polypeptide according to claim 2, wherein the said *Helicobacter pylori* polypeptide FlaA comprises the amino acid sequence set forth as SEQ ID NO: 2, for use in inducing a protective immune response to *Helicobacter pylori* infection.  
15
4. The polypeptide according to claim 1 which comprises the *Helicobacter pylori* polypeptide FlaB, or a modified form of the said polypeptide retaining functionally equivalent antigenicity, for use in inducing a protective immune response to *Helicobacter pylori* infection.  
20
5. The polypeptide according to claim 4, wherein the said *Helicobacter pylori* polypeptide FlaB comprises the amino acid sequence set forth as SEQ ID NO: 4, for use in inducing a protective immune response to *Helicobacter pylori* infection.
- 25 6. A vaccine composition for inducing a protective immune response to *Helicobacter pylori* infection, comprising an immunogenically effective amount of a polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide, optionally together with a pharmaceutically acceptable carrier or diluent.
- 30 7. The vaccine composition according to claim 6, wherein the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaA.



8. The vaccine composition according to claim 7, wherein the said *Helicobacter pylori* polypeptide FlaA comprises the amino acid sequence set forth as SEQ ID NO: 2.
- 5 9. The vaccine composition according to claim 6, wherein the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaB.
10. The vaccine composition according to claim 9, wherein the said *Helicobacter pylori* polypeptide FlaB comprises the amino acid sequence set forth as SEQ ID NO: 4.
- 10 11. The vaccine composition according to any one of claims 6 to 10, in addition comprising an adjuvant.
12. The vaccine composition according to claim 11 wherein the adjuvant is a  
15 pharmaceutically acceptable form of cholera toxin.
13. The vaccine composition according to any one of claims 6 to 12 for use as a therapeutic vaccine in a mammal, including man, which is infected by *Helicobacter pylori*.
- 20 14. The vaccine composition according to any one of claims 6 to 12 for use as a prophylactic vaccine to protect a mammal, including man, from infection by *Helicobacter pylori*.
- 25 15. Use of a polypeptide comprising at least one *Helicobacter pylori* flagellin polypeptide in the manufacture of a composition for the treatment or prophylaxis of *Helicobacter pylori* infection.
- 30 16. The use according to claim 15, wherein the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaA.



17. The use according to claim 16, wherein the said *Helicobacter pylori* polypeptide FlaA comprises the amino acid sequence set forth as SEQ ID NO: 2.
- 5 18. The use according to claim 15, wherein the said polypeptide comprises the *Helicobacter pylori* polypeptide FlaB.
19. The use according to claim 18, wherein the said *Helicobacter pylori* polypeptide FlaB comprises the amino acid sequence set forth as SEQ ID NO: 4.
- 10 20. The use according to any one of claims 15 to 19, wherein the said composition comprises a vaccine effective in eliciting a protective immune response against *Helicobacter pylori*.
- 15 21. A method of eliciting in a mammal a protective immune response against *Helicobacter pylori* infection, said method comprising the step of administering to the said mammal an immunologically effective amount of a vaccine composition according to any one of claims 6 to 12.
- 20 22. The method according to claim 21 wherein the said mammal is a human.