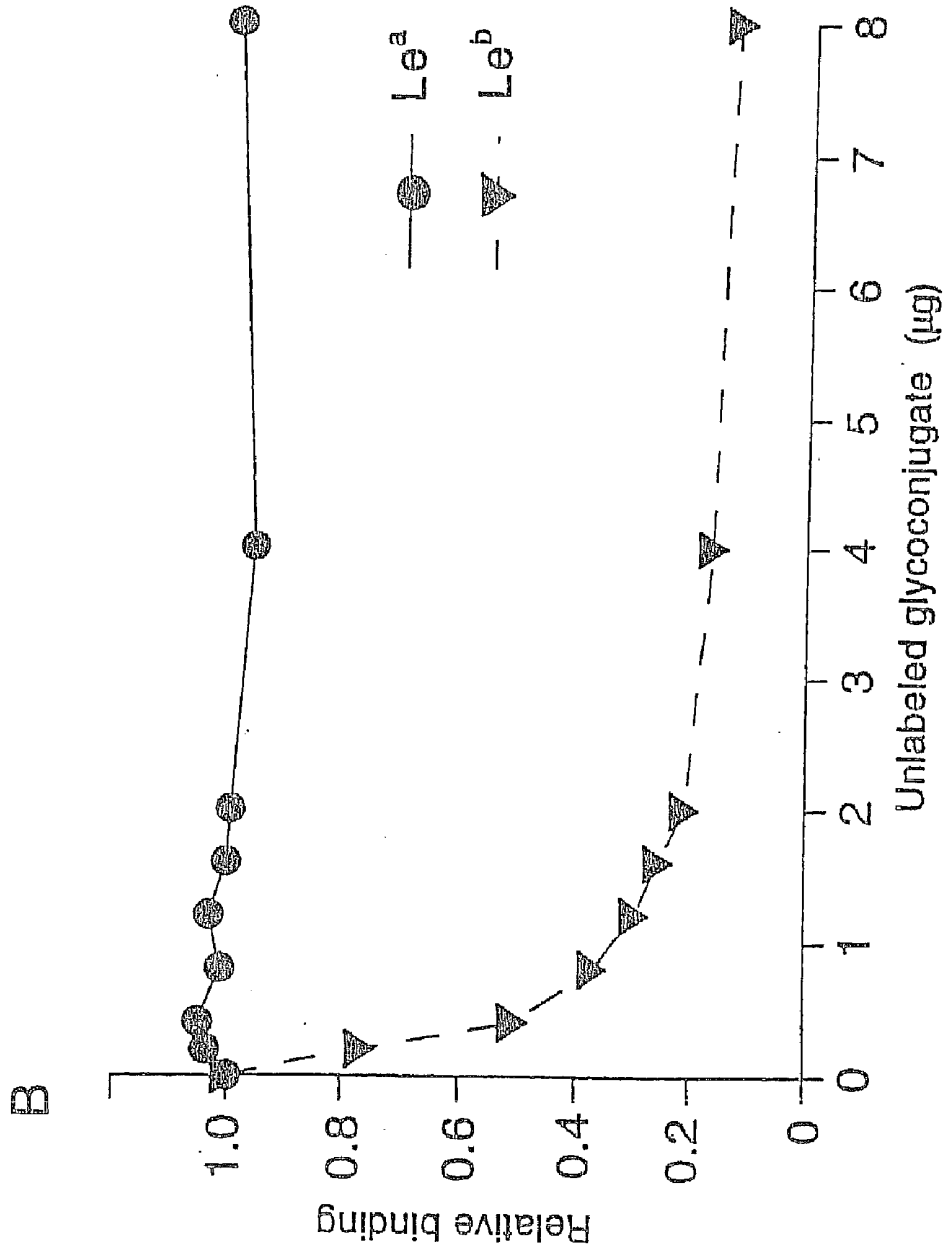


**FIG 1A**



**FIG 1B**

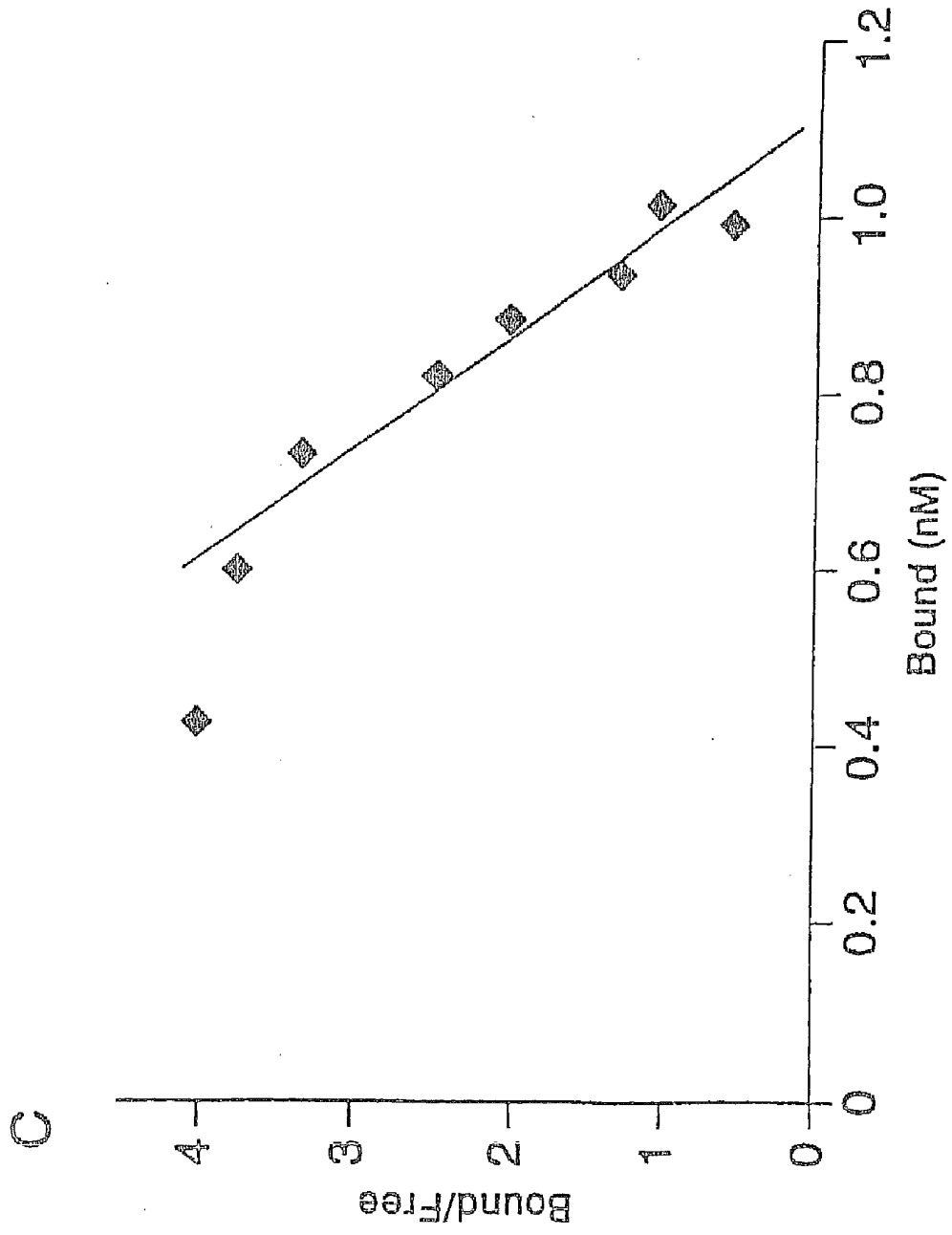


FIG 1C

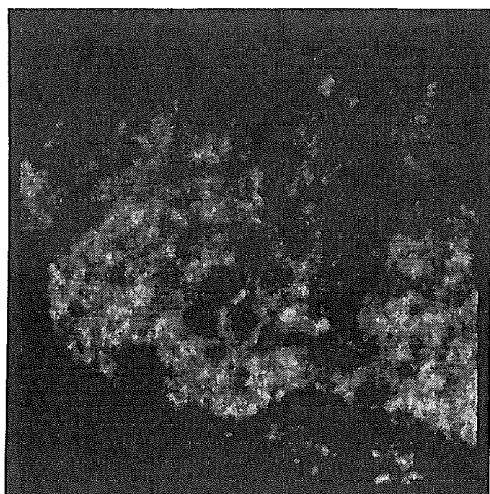


Fig.2A

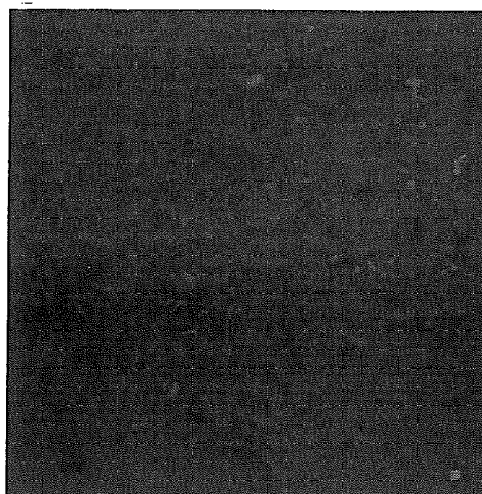


Fig.2B

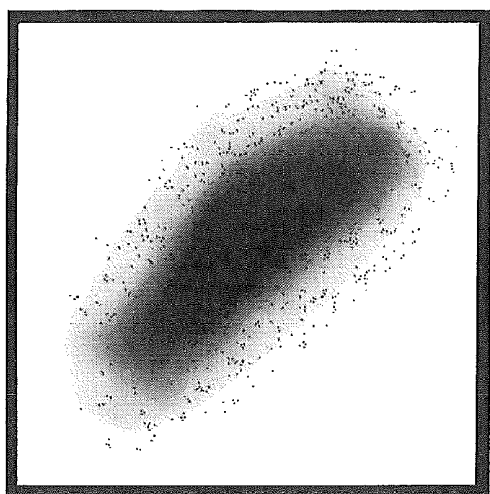


Fig.2C

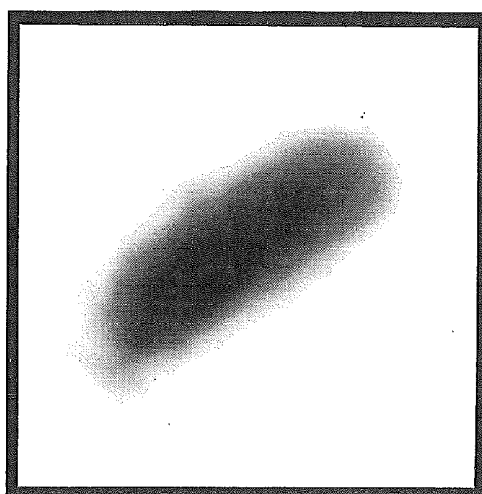


Fig.2D

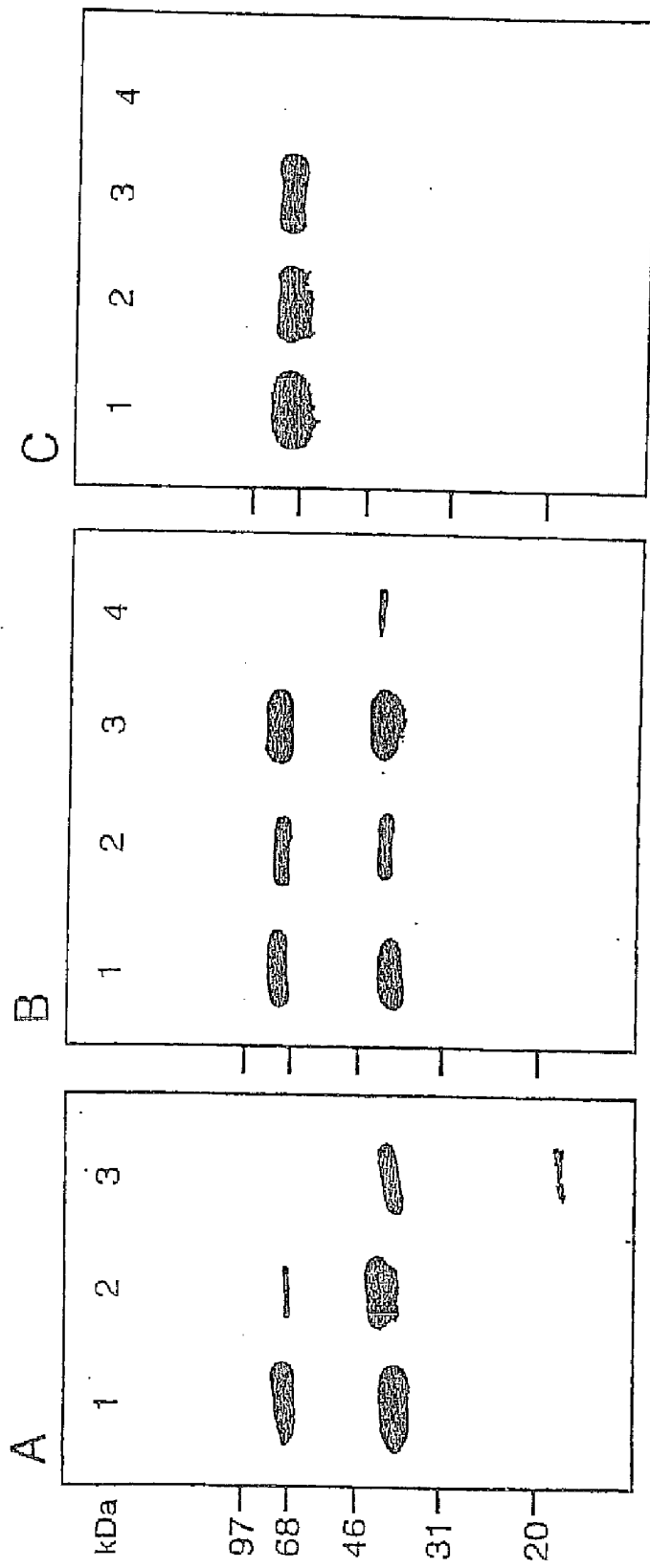
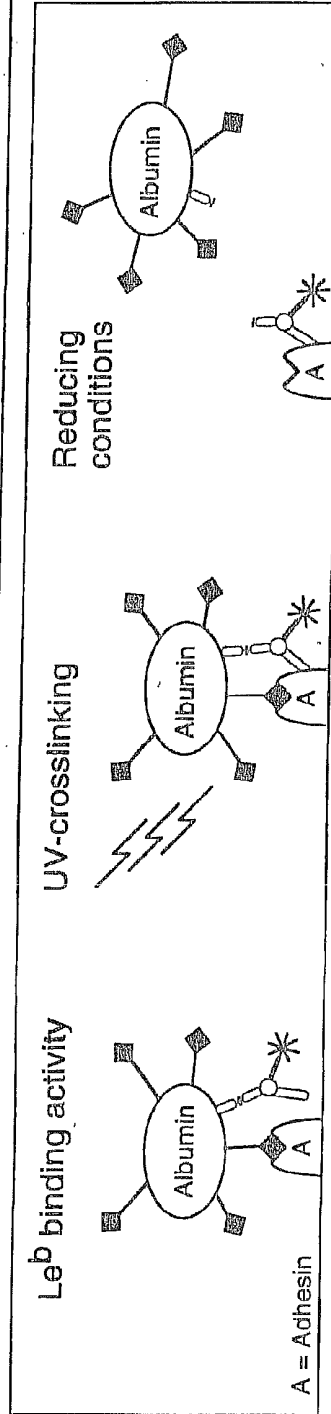
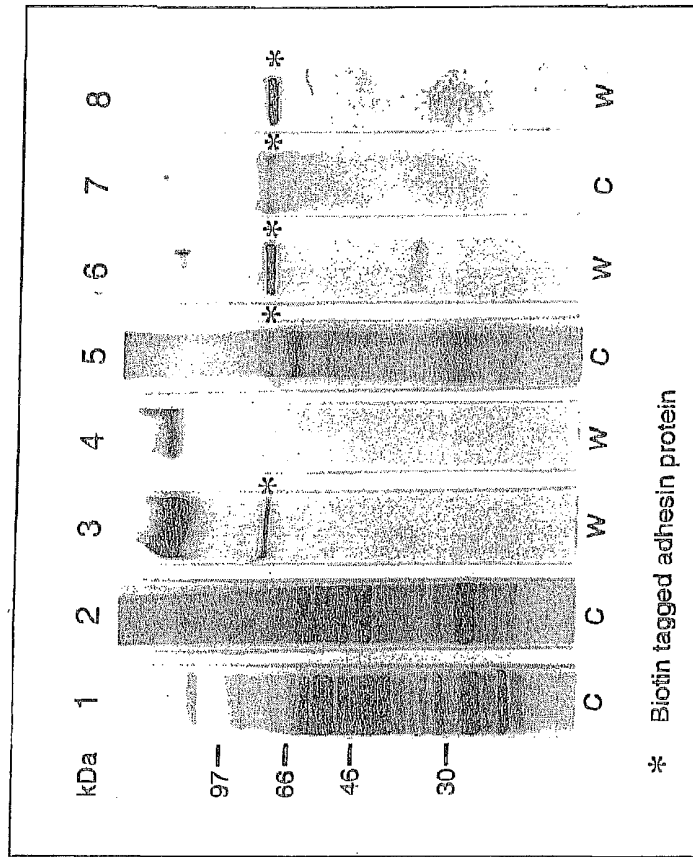
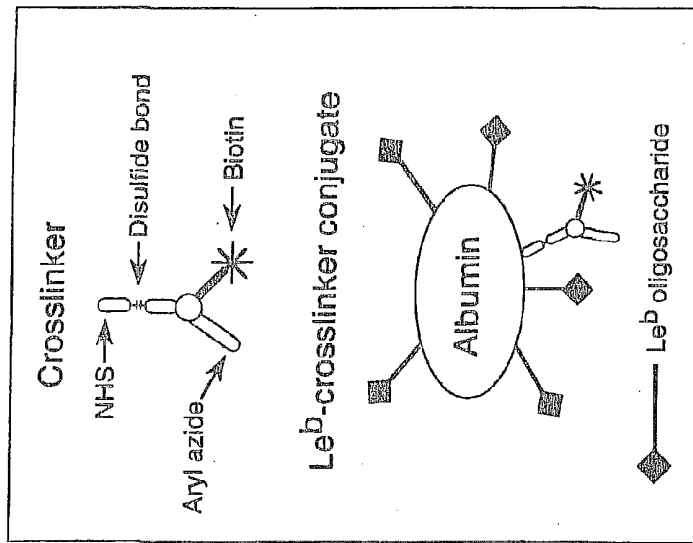


Fig. 3





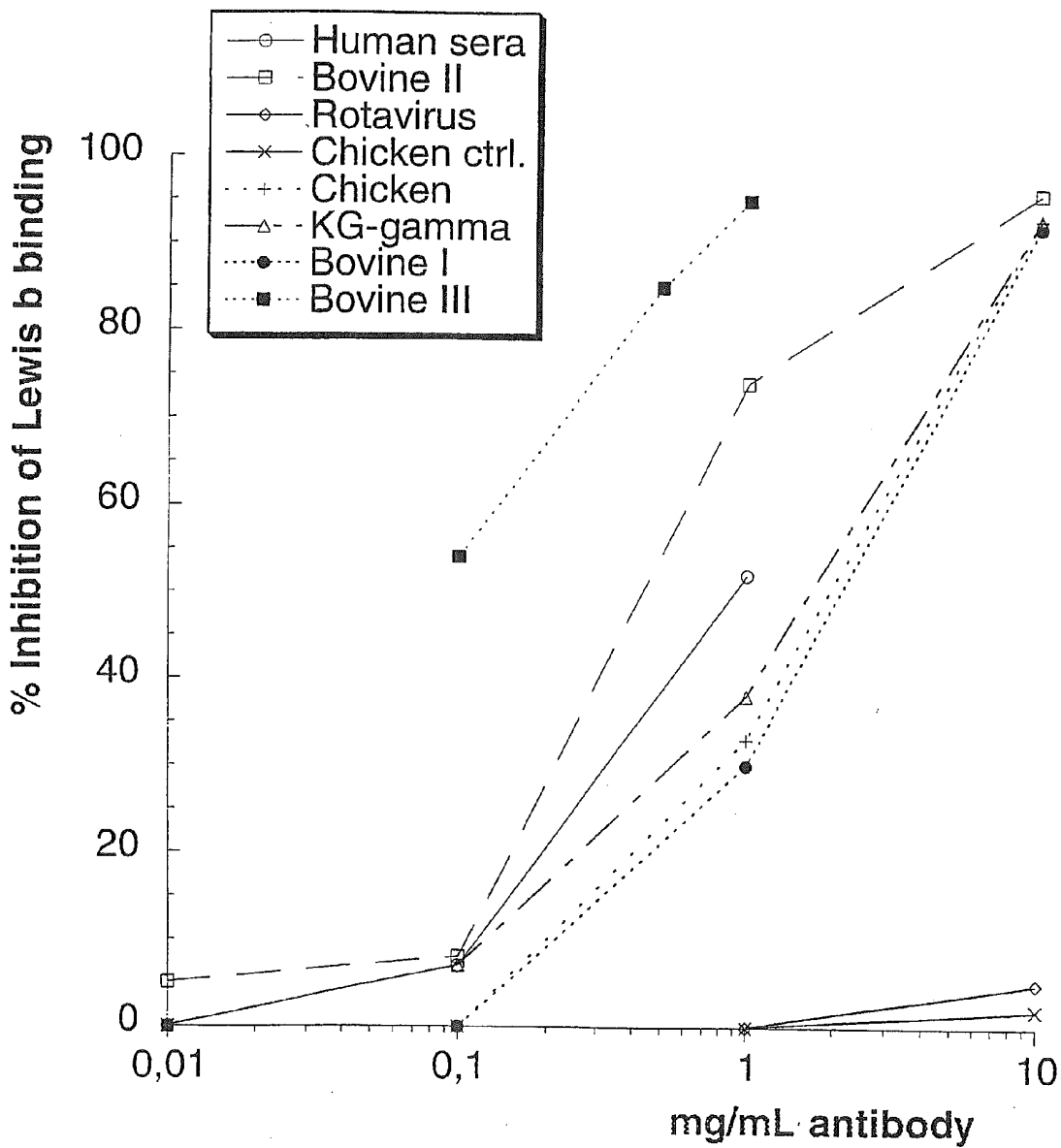


Fig. 6

Antibody preparations used for Western blot detection of the BabA adhesin and *H. pylori* protein antigens;

Human Sera      Chicken Antibody      Bovine Antibodies I II III II/III

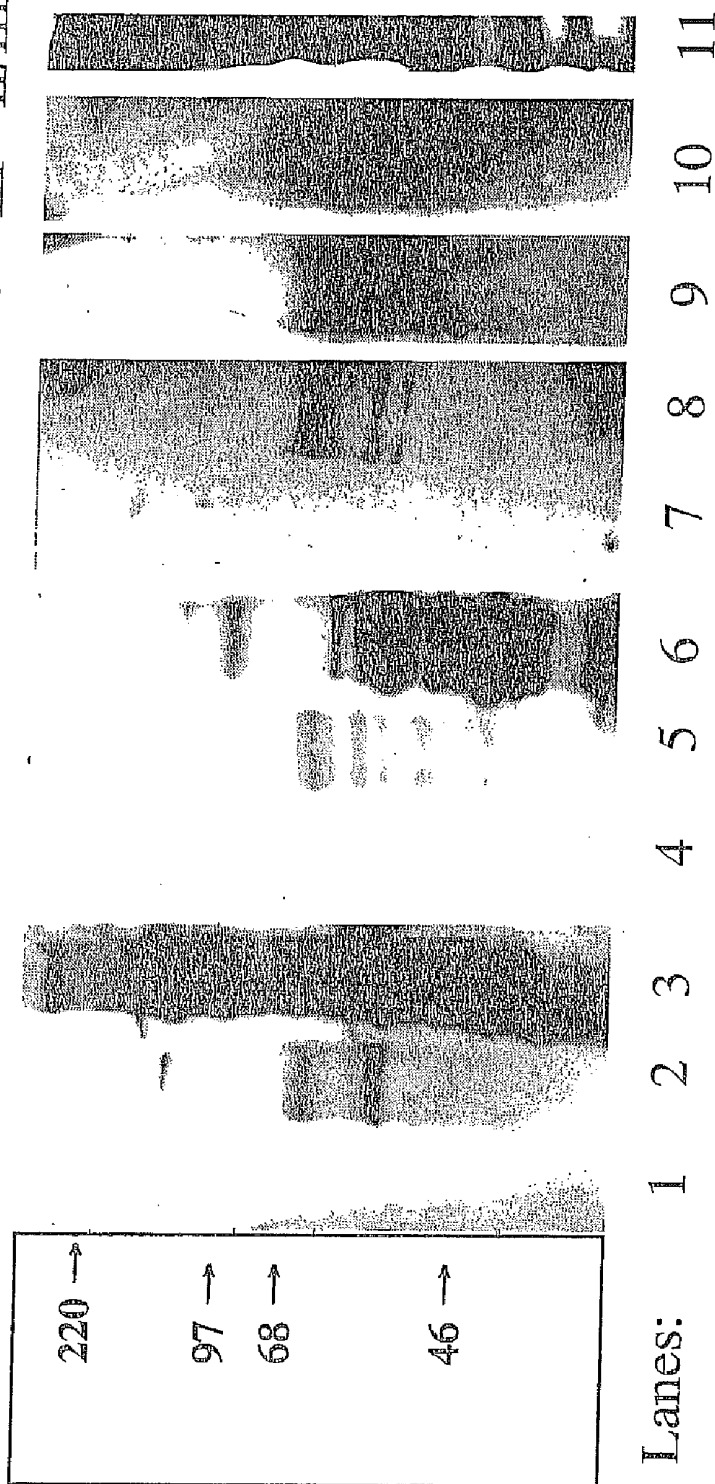


Fig.7

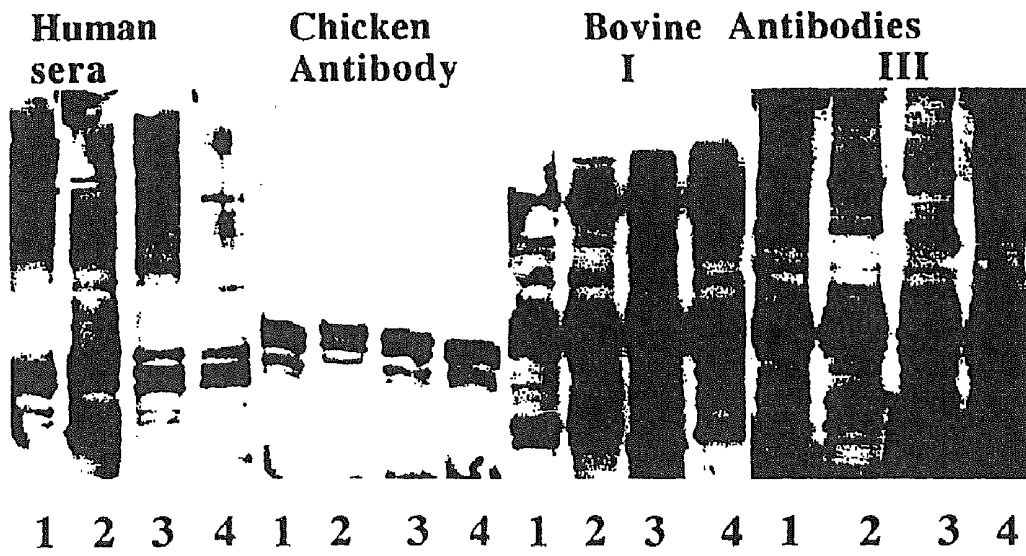


Fig.8

## HELICOBACTER PYLORI ADHESIN BINDING GROUP ANTIGEN

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. patent application Ser. No. 10/761,201, filed Jan. 22, 2004, which is a divisional of U.S. patent application Ser. No. 09/202,178 (now issued as U.S. Pat. No. 6,709,656), which is the U.S. National Stage of International Application No. PCT/SE97/01009 filed Jun. 10, 1997, which claims priority to Swedish Application No. 9602287-6 filed Jun. 10, 1996 and Swedish Application No. 9701014-4 filed Mar. 19, 1997. All of the preceding applications are incorporated by reference herein in their entirety.

### FIELD OF THE INVENTION

[0002] The present invention relates to materials and methods for prevention, treatment and diagnosing of infections caused by *Helicobacter pylori*. More specifically the present invention relates to polypeptides and antibodies useful in vaccines for the treatment and prevention of pathologic infections caused by *Helicobacter pylori* strains. The present invention specifically relates to a bacterial blood group antigen binding adhesin (BAB-adhesin). The present invention further relates to polynucleotides useful for the recombinant production of said polypeptides and for use in immunisation therapies. In addition, it relates to polypeptides, antibodies, and polynucleotides used for the detection of said bacteria.

[0003] The present invention further relates to new immunoglobulins, which exhibit specific activity to a blood group binding adhesin, expressed by *Helicobacter pylori*, methods for the production of said immunoglobulins, their isolation and use. The present invention further relates to the treatment and prevention of *H. pylori* induced infections in the gastrointestinal tract.

### BACKGROUND OF THE INVENTION

[0004] *Helicobacter pylori* is a causative agent for acid peptic disease and the presence of this organism is highly correlated to the development of gastric adenocarcinoma. Bacterial adherence to the human gastric epithelial lining was recently shown to be mediated by fucosylated blood group antigens.

[0005] Recent research has focused on the role of *Helicobacter pylori* in the development of ulcers in the gastric mucosa. Recent findings show a strong connection between *H. pylori* and chronic, active gastritis and gastric ulcers. Furthermore, there appears to be a strong correlation between ventricular cancer and gastric ulcers. Traditional treatment of gastric ulcers has involved gastric resection, the administration of bismuth compositions, the administration of H<sub>2</sub>-blockers and the administration of pH-buffering agents, to mention a few examples.

[0006] More recently, various forms of treatment have been supplemented with the administration of antibiotics. One problem with presently known treatments is the risk for treatment failure. Furthermore, not only do microbes develop antibiotic resistance, the antibiotics administered often upset the natural balance of benign microbes, colonising the intestinal tract. This leads to diarrhea and other signs of intestinal discomfort, in addition to destabilising the benign flora in the

intestines. Other treatments, e.g. H<sub>2</sub>-blockers often require life-long medication to prevent the recurrence of disease.

[0007] The foregoing, together with the fact that *H. pylori* is very widely spread among humans—according to a conservative estimate approximately 60% of all adult humans in the industrialised countries are infected—makes the diagnosing, prevention and treatment of *H. pylori* infections an urgent task.

[0008] Further, the fact that developing countries frequently lack the resources for conventional treatment of gastric ulcers further underlines the importance of finding new ways of treatment and prevention of *H. pylori* induced infections. It is obvious, for many reasons, that disease prevention with vaccines is a preferable mode. A vaccine would provide an easily administered and economical prophylactic regimen against *H. pylori* infections. An effective vaccine against *H. pylori* is nevertheless presently lacking.

### STATE OF THE ART

[0009] *H. pylori* colonises the human gastric mucosa, in an equilibrium between adherence to the epithelial surface mucous cells and the mucous layer lining the gastric epithelium. Once infected, bacteria seems to colonise for a lifetime. Attachment to the epithelial lining protects the bacteria from the anti-microbial effects of the acidic gastric juice of the stomach lumen, as well as from physical forces such as peristalsis. For survival in this hostile ecological niche, *H. pylori* has developed a battery of virulence factors; such as production of the enzyme urease that buffers the micro-environment around the bacteria and the polar flagellae to ensure high motility, a prerequisite in an ecological niche where the turnover of the mucous layer is in the range of hours. A subset of *H. pylori* strains produces the vacuolating cytotoxin, VacA, and the cytotoxin associated antigen CagA.

[0010] Attachment is essential for colonisation of the epithelial lining and bacteria express surface associated adhesion molecules that recognise specific carbohydrate or protein receptors on the cell surfaces or mucous lining. The specificity in this interaction in combination with the genetically regulated receptor distribution results in a restricted range of cell lineages and tissues available for colonisation. Several putative receptor structures have been described for *H. pylori*, such as the hemagglutinin-sialic acid, sulphated glycoconjugates and sulphatides. Recently, the fucosylated blood group antigens H-1 and Lewis<sup>b</sup> were described (Borén et al., Science, 262, 18921993), mediating specific adherence of *H. pylori* to human and rhesus monkey gastric surface mucous cells in situ. The H-1 and Lewis<sup>b</sup> antigens are part of the blood group antigens that define blood group 0 in the ABO system.

[0011] Surface-exposed proteins are often constituents of the outer membrane. The outer membrane has a structural role and acts as a selective barrier, determining what enters the cell and what molecules are secreted. One class of outer membrane proteins are called porins, and create hydrophilic pores through the outer membrane where specific metabolites, such as sugar molecules, can cross. Recently the finding of a number of outer membrane proteins in *H. pylori*, was reported, which proteins were suggested to constitute a family of porin proteins.

[0012] The BAB adhesin has previously been identified and shown to be localised on the bacterial surface of *H. pylori* (SE 9602287-6). The blood group binding activity was shown to be pH dependent and the present inventors present evidence

that the binding affinity to the Lewis<sup>b</sup> receptor reveals a high equilibrium constant. For the purification of the BAB adhesin, a crosslinker-labelled receptor conjugate was used in order to mediate specific transfer of biotin to the adhesins on the bacterial surface. Thereafter the biotin-labelled adhesin could be extracted by streptavidin coated magnetic beads. Determination of the amino terminal amino acid sequence of the purified BAB adhesin exhibit homologies to outer membrane proteins of *H. pylori* porins.

**[0013]** Intensive research has been directed to the immunological treatment and prevention of *H. pylori* induced infections. EP 0 484 148 (Ando & Nakamura) describes a method for treating and/or preventing upper gastrointestinal disease in mammals, said method comprising orally administering to a patient in need thereof an effective amount of a pharmaceutical composition comprising anti-*Helicobacter pylori* polyclonal immunoglobulins and a pharmaceutically acceptable carrier. Said description further dwells on the combination of said treatment in combination with the administration of antibiotics. As the method of producing said polyclonal antibodies, EP 0 484 148 describes the isolation and purification of anti-*H. pylori* immunoglobulins from the sera and milk of mammals. *H. pylori* itself was not found in the stomachs of cows, goats, sheep, swine or horses, according to EP 0 484 148, but it was assumed that these animal species have colonizing microorganisms with antigenic determinants similar to those of *H. pylori* because they have immunoglobulins which cross-react to strains of *H. pylori* found in humans. Preferably, according to EP 0 484 148, large mammals, e.g. pregnant cows, are immunized with whole cells of *H. pylori* and the immunoglobulins subsequently extracted from the milk or colostrum. In the immunization experiments, NCTC Strain 11362 and clinical isolate *H. pylori* No. 153 were used to trigger the production of immunoglobulins. On the other hand, NCTC Strain 11637 was used for analysing purposes. Immunization is claimed to yield an anti-*H. pylori* titer in the milk of such magnitude, that daily doses of 0.01-0.1 g/day immunoglobulin composition, are sufficient for successful therapy. The claimed interval of 0.01-0.1 g/day is however not supported by the experiments presented by Ando & Nakamura and so low doses have hitherto not proven efficient in clinical tests. The doses actually used in example 5 and 7 are in the order of magnitude of 1 g/day, i.e. 10-fold the upper limit of the given interval. Furthermore, it is very unlikely, that unspecific immunoglobulin mixtures as those manufactured by Ando & Nakamura, would be effective in claimed doses as similar doses are ineffective against other gastrointestinal pathogens. The simultaneous administration of antibiotics, extensively discussed in the description, underlines the insufficiency of the disclosed immunoglobulins.

**[0014]** EP 0 469 359 (Cordle & Schaller) likewise describes the immunization of mammals, preferably pregnant cows, with formalin killed *H. pylori* bacteria (ATCC Strain 26695). Anti-*H. pylori* polyclonal antibodies were isolated and purified from the milk and finally fed to piglets, in amounts of about 0.5 g immunoglobulins, three times daily. The results were assessed by determination of the number of biopsy specimens, which were positive for Gram-negative bacteria after the trial. Gram-negative bacteria was found in 78% of the piglets fed a non-immune nutrient but only (sic!) in 35% of the piglets fed a nutrient containing so called specific anti-*H. pylori* antibodies.

**[0015]** Anti-*H. pylori* polyclonal antibodies, effective to cause aggregation of *H. pylori*, have thus been administered

orally as a regimen in the treatment and prevention of *H. pylori* induced infections in the gastrointestinal tract. Nevertheless, as also noted in EP 0 484 148 A1, it is still not clear, how many antigenic determinants are present on the surface of *H. pylori*. The occurrence of a wide variety of *H. pylori* strains, makes questionable the practical efficiency of any polyclonal immunological therapy according to the state of the art. Immunization using whole bacteria will always trigger a highly polyclonal immunresponse with a low level of antibodies against a given antigenic determinant. This is underlined e.g. by the results presented by Cordle & Schaller, where, although the number of *Helicobacter* positive biopsies were reduced, complete cure was not obtained through the treatment according to their invention.

**[0016]** It is notable, that the dose of immunoglobulin needed for oral prophylaxis or therapy has not yet been clearly defined. In a normal human adult, approximately 5 g IgA is produced and secreted at mucosal surfaces each day. Obviously, doses of this magnitude are economically and practically unfeasible for large-scale therapy or prophylaxis. In studies on the effect of oral immunoglobulin on rotavirus infection, daily doses in the interval of 600 to 9000 mg have been tried in clinical tests. Successful intervention has also been reported when treating *H. pylori* and cryptosporidial infections with daily administrations of 3 to 15 g immunoglobulin from immunized cows (Hammarström et al., Immunol Rev, 139 (1994) 43-70). Generally speaking, all studies hitherto point to the necessity of using high doses of immunoglobulins when trying to combat an ongoing infection. The need for more specific immunoglobuline preparations, allowing the use of smaller doses, is thus an urgent one.

**[0017]** To maximize the potency of an immunological regimen for the treatment and prevention of *H. pylori*, it is of great importance to find a specific conserved antigenic determinant, which plays a central role for the pathogenicity of *H. pylori*. Using such an antigenic determinant would make it possible to produce highly specific and therapeutically efficient novel polyclonal and/or monoclonal immunoglobulin preparations.

#### SUMMARY OF THE INVENTION

**[0018]** The above problem of providing specific, cost-efficient and therapeutically superior immunoglobulin preparations for the treatment and prevention of *H. pylori* has now been solved through the composition and methods according to the attached patent claims. The present inventors have now surprisingly shown, that highly specific and therapeutically efficient polyclonal and/or monoclonal immunoglobulin preparations can be provided through the immunization of an animal with an adhesin protein, specific for *H. pylori*. Said adhesin protein is characterized already in the priority applications SE 9602287-6 and SE 9701014-4, which hereby are referred to in their entirety. The invention will now be described in closer detail with reference to the attached, non-limiting figures and examples.

**[0019]** One objective of the present invention was to further purify and characterize the *H. pylori* blood group antigen binding (BAB) adhesin to make possible the development of methods and materials for specific and selective diagnosing and treatment of *H. pylori* induced infections and related diseases and the development of said methods and materials. A further and equally important objective was to determine the DNA sequences of the genes involved in the expression of this protein. These objectives were fulfilled through the pro-

tein specified in claim 1, the DNA disclosed in claim 13 and 14 and the methods and materials specified in the subsequent claims. The DNA sequences are attached as Appendix 1 and 2, disclosing the babA and babB sequences, respectively. The full protein sequence is disclosed in Appendix 3.

#### DESCRIPTION OF THE FIGURES

[0020] FIG. 1 A) illustrates the bacterial binding to soluble blood group antigens. *H. pylori* strains were incubated with <sup>125</sup>I-labeled blood group antigen glycoconjugates and bound <sup>125</sup>I-activity was measured (Note the absence of blood group antigen binding shown for strains MO19 and 26695.).

[0021] FIG. 1 B) illustrates a receptor displacement assay. Strain CCUG 17875 was first incubated with 10 ng <sup>125</sup>I-labeled Le<sup>b</sup> antigen glycoconjugate and the complex was then challenged (1 h) with an excess of unlabeled Le<sup>b</sup> or Le<sup>a</sup> glycoconjugate, before the <sup>125</sup>I-activity in the bacterial pellet was measured. Concentrations of the unlabeled glycoconjugate ranged from 50 ng to 8 μg and C) shows the results of a Scatchard analysis of the *H. pylori*-Le<sup>b</sup> antigen interaction. Bacterial binding to the Le<sup>b</sup> glycoconjugate was titrated to an affinity constant (K<sub>a</sub>) value of 8×10<sup>-10</sup> M<sup>-1</sup> (13).

[0022] FIG. 2: Upper panel: Prevalence of the BabA adhesin in the bacterial population. Cells of strain CCUG 17875 were incubated with biotinylated Le<sup>b</sup> (A) or Le<sup>a</sup> (B) glycoconjugate. Bound biotinylated Lewis-conjugate was detected with FITC-labeled streptavidin (green fluorescence) and bacteria were counterstained with propidium iodine (red fluorescence). Lower panel: Localisation of the BabA adhesin. For electron microscopy (15) cells of strain CCUG 17875 were incubated with biotinylated Le<sup>b</sup> (C) or Le<sup>a</sup> (D).

[0023] FIG. 3 shows the characterization of the molecular weight of the BabA adhesin by the use of receptor overlay analysis (A, B) and receptor activity directed affinity tagging of BabA (C).

[0024] FIG. 4 shows receptor activity directed affinity tagging and protein purification of the BabA adhesin.

[0025] FIG. 5 shows the translated amino acid sequences for the babA and babB genes, corresponding to the N-terminal domain of the BabA adhesin.

[0026] FIG. 6 shows the procentage inhibition of *H. pylori* binding to <sup>125</sup>I-labeled Lewis b antigen for different preparations as a function of the antibody titre.

[0027] FIG. 7 shows a Western blot detection of the BabA adhesin by the different antibody preparations.

[0028] FIG. 8 shows four Western blot analyses of *H. pylori* proteins by the different antibody preparations.

#### DESCRIPTION OF THE INVENTION

[0029] The blood group antigen binding adhesin, BabA, has now been biochemically characterized and purified by a novel technique, receptor Activity Directed Affinity Tagging (Retagging). Two genes, babA and babB were found to code for two different but very similar proteins. The present invention thus comprises a novel blood group antigen binding adhesin according to claim 1 and the subsequent claims. The DNA sequences are disclosed in appendices 1 (babA) and 2 (babB). The protein sequences is disclosed in appendix 3. The invention also includes any pharmaceutical composition comprising said adhesin protein and/or fractions thereof. Examples of such pharmaceutical compositions are for example medicaments for the prevention or treatment of *Helicobacter pylori* induced gastritis, gastric and duodenal ulcers

and gastric adenocarcinoma. Optionally said pharmaceutical composition additionally encompasses pharmaceutically acceptable excipients.

[0030] Further, the present invention comprises the BAB-adhesin gene or genes for expression of an adhesin protein according to the invention. Said invention also comprises a novel method for the isolation and purification of said adhesin. The disclosed genes are contemplated to function as a cassette system, the organism alternating between these to avoid immunity in the host. It is very likely, that homologies of the disclosed sequences exist and additionally supplement said cassette function in other strains of *H. pylori*. Also, genes corresponding to a homology of the first 40 amino acids or genes, corresponding to a homology of the last, about 300 amino acids, can function to this effect. It is further highly likely, that *Helicobacter pylori* is able to switch between several genes, similar to the disclosed genes, in a so-called cassette system.

[0031] The invention additionally comprises monospecific antisera produced using the novel adhesin protein and/or fractions thereof. Said monospecific antisera is preferably produced according to any suitable, conventional method for producing monospecific antisera in vitro or in vivo, e.g. by inoculating a suitable animal. Such methods are familiar to a person skilled in the art. Antibodies raised in a suitable animal or in the patient to be treated, can subsequently be administered locally, e.g. orally to the patient.

[0032] The invention further comprises the use of said monospecific antisera for the manufacturing of a test kit for quantitative or qualitative determinations of adhesin protein or fractions thereof in cells, tissues or body fluids.

[0033] The invention further comprises the use of said adhesin protein or corresponding DNA for use in therapy or immunisation and/or in the manufacture of compositions for said uses. The invention specifically encompasses the use of said DNA for immunisation therapy and for the manufacture for compositions for such therapy. Preferably, in an immunisation therapy where said composition is administered orally to a patient, the adhesin protein, fractions thereof or said DNA is administered in combination with a pharmaceutically suitable immunostimulating agent. Examples of such agents include, but are not limited to the following: cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and similar agents. The composition according to the present invention can further include conventional and pharmaceutically acceptable adjuvants, familiar to a person skilled in the art of immunisation therapy. Preferably, in an immunisation therapy using the inventive DNA or fractions thereof, said DNA is preferably administered intramuscularly, whereby said DNA is incorporated in suitable plasmide carriers. An additional gene or genes encoding a suitable immunostimulating agent can preferably be incorporated in the same plasmide.

[0034] Said immunisation therapies are not restricted to the above-described routes of administration, but can naturally be adapted to any one of the following routes of administration: oral, nasal, subcutaneous and intramuscular. Especially the oral and nasal methods of administration are promising, in particular for large-scale immunisations.

[0035] The present inventors have surprisingly shown, that highly specific and therapeutically efficient polyclonal and/or monoclonal immunoglobulin preparations can be provided through the immunisation of an animal with an adhesin protein or fractions thereof, specific for *H. pylori*. When consid-

ering immunisation against *H. pylori*, it is worth noting that the infection is known to be lifelong despite a vigorous immune response in the gastric mucosa. An increased local production of IgA in the mucosa is not necessarily enough and the administration of monospecific antibodies directed against a central virulence factor, such as the adhesin according to the present invention, may constitute a more effective approach.

**[0036]** The term “immunisation” refers here to a method for inducing a continuous high level of antibody and/or cellular immunoresponse. The term “animal” here preferentially denotes any member of the subphylum Vertebrata, a division that includes all animals, including mammals, which are characterized by a segmented bony or cartilaginous spinal column. All vertebrates have a functional immune system and respond to antigens by producing antibodies. The term “protein” is used here to denote a naturally occurring polypeptide and the term “polypeptide” is used here in its widest meaning, i.e. any amino acid polymer (dipeptide or longer) linked through peptide bonds. Accordingly the term “polypeptide” comprises proteins, oligopeptides, protein fragments, analogues, muteins, fusion proteins and the like. The term “antibody” as used in this context includes an antibody belonging to any of the immunological classes, such as immunoglobulins A, D, E, G or M. Of particular interest are nevertheless immunoglobulin A (IgA) since this is the principle immunoglobulin produced by the secretory system of warm-blooded animals. However, in cow colostrum, the main antibody class is IgG 1.

**[0037]** Borén et al. have recently isolated and characterized a Lewis<sup>b</sup> binding protein with a molecular weight of about 73500 Da (See the priority applications SE 9602287-6 and SE 9701014-4, which are referred to in their entirety). This adhesin protein is thought to be a conserved structure and specific for pathogenic strains of *H. pylori*. Said protein is specific for at least one of the *H. pylori* strains included in the following group: CCUG 17875, NCTC 11637, A5, P466, G109, G56, Ba 185, Ba 99, 931 and 932.

**[0038]** This adhesin protein or immunologically effective fractions thereof are characterized in that the following amino acid sequence is included:

EDDGFYTSVGYQIGEAQMV

or homologues thereof.

**[0039]** The following DNA sequence or homologues thereof is included in DNA for expression of said adhesin protein or fractions thereof:

5' -GAAGACGACGGCTTTACACAAGCGTAGGCTATCAAATCGGTGAAGC  
CGCTCAAATGGTA- 3'

**[0040]** According to one embodiment of the invention, a pregnant mammal, preferably a cow or another suitable domestic animal, is immunised with said Lewis<sup>b</sup> binding adhesin protein or fractions thereof. The adhesin protein or fractions thereof is/are preferably injected intramuscularly or subcutaneously in the chosen animal, optionally together with suitable adjuvants. Examples of such adjuvants include, but are not limited to immunostimulating agents such as cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and similar, conventional agents, such as classical adjuvants including mineral and vegetable oils. Sub-

sequent to the regimen of immunization, comprising a necessary amount of doses, including so called booster-doses, over a time span allowing for optimal immunoglobulin expression, milk or sera is collected from said animal. Preferably the cow colostrum, which is specially high in immunoglobulins, is collected. The specific immunoglobulin fraction according to the present invention is then separated and purified in a conventional manner, e.g. including separation of fats, protein precipitation and concentration by ultrafiltration.

**[0041]** According to another embodiment of the invention, a bird, preferably a chicken or another suitable domestic bird, is immunized with said Lewis<sup>b</sup> binding adhesin protein or fractions thereof. The adhesin protein or fractions thereof is preferably injected intra-muscularly or subcutaneously in the chosen bird, optionally together with suitable adjuvants. Examples of such adjuvants include, but are not limited to immunostimulating agents such as cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and similar, conventional agents, such as classical adjuvants including mineral and vegetable oils. Subsequent to the regimen of immunization, comprising a necessary amount of doses, including so called booster-doses, over a time span allowing for optimal immunoglobulin expression, sera or eggs is/are collected from said animal. Preferably the egg yolk, which is specially high in immunoglobulins, is collected. The specific immunoglobulin fraction according to the present invention is then separated and purified in a conventional manner, e.g. including protein precipitation and ultrafiltration. Alternatively, the egg yolk being of high nutritional value in addition to containing a high titer of specific antibodies according to the present invention, can be administered as such.

**[0042]** According to a preferred embodiment of the present invention, monoclonal immunoglobulin is produced by establishing transgenic animals. Said transgenic animals can be chosen from the following group of species: mammals, e.g. cow, goat and rabbit, and birds: e.g. chicken, duck, turkey. The mammal most preferably used is cow and the most preferable bird is chicken. Further developments of transgenic animals such as mice and rats could also offer new possibilities. The choice of animal is naturally governed by availability and local adaptation.

**[0043]** According to one embodiment, a stock of transgenic animals according to the present invention, adapted to the local conditions, are kept locally, e.g. in villages in developing countries to function as local units for the production of immunoglobulins for oral administration. For example transgenic cows, goats or chicken are suitable for this purpose and preferably chicken are used. Consumption of the milk or preferably the eggs, produced by the transgenic animals, can help to eradicate presently very difficult infectious diseases, e.g. diseases caused by *H. pylori*.

**[0044]** According to yet another embodiment of the present invention, monoclonal antibodies can be produced using the hybridoma method. The hybridoma method is well known to a skilled worker in the field of biochemistry and it is described e.g. in Galfre, G. And Milstein, C., Preparation of monoclonal antibodies: strategies and procedures (Methods in Enzymology, 73:3-46, 1981). A suitable host animal is immunized with the Lewis<sup>b</sup> binding adhesin protein or fractions thereof. When the immunization is accomplished, the animal is sacrificed, spleen cells collected and fused with cells from a neoplastic cell line, preferably myeloma cells. By choosing the growth conditions, the successfully fused hybridoma cells

can be selected. The monoclonal antibodies produced by the hybridoma cell line can then be administered orally in a regimen for treatment and/or prevention of *H. pylori* infections.

**[0045]** Preferably the polyclonal and/or monoclonal antibodies are purified prior to administration and, more preferably, admixed with pharmaceutically suitable carriers and/or adjuvants. Examples of suitable carriers are saline, pharmaceutically acceptable fats, oils, carbohydrates and proteins. The carrier or carriers is/are preferably chosen so that the solubility and absorption of the immunoglobulin in the mucus layer lining the stomach is enhanced. Using suitable adjuvants the stability, therapeutic efficiency and nutritional value of the composition can be improved. To improve stability under storage, the immunoglobulin composition can be lyophilized. Regardless of the exact preparation and formulation, it is of central importance to avoid denaturing the immunoglobulins.

**[0046]** The higher specificity, exhibited by the immunoglobulin preparation of polyclonal and/or monoclonal antibodies according to the invention, makes it possible use substantially lower doses compared to those presently used, thus lowering the cost and improving the availability of the treatment. The use of specific, monoclonal antibodies can make it possible to further lower the doses. The doses are in all cases a function of the antibody titer of the preparation. A high titer naturally allows the use of lower doses.

**[0047]** According to one embodiment of the invention, an immunoglobulin preparation is manufactured as follows: an animal is immunized with a Lewis<sup>b</sup> binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*, the immunoglobulin fraction is isolated from an excretion of said animal and subsequently purified. The purified immunoglobulin composition is admixed with suitable carriers and adjuvants to form an immunoglobulin preparation for the prevention or treatment of *H. pylori* infections. In cases where the antibody titer is sufficiently high and the other constituents of the immunoglobulin composition isolated from the animal are harmless, for example in the case of colostrum from immunized cows or egg yolk from immunized chicken, there is always the option of administering the colostrum or egg yolk to the patient without any further treatment of the colostrum or egg yolk.

**[0048]** The immunoglobulin composition according to the invention is preferably administered orally to the patient, in the smallest therapeutically or prophylactically effective dose. Presently conceived are doses in the interval of 0.1 to 1000 mg/day, preferably in the interval of 0.1 to 100 mg/day. The chosen doses naturally depend on the antibody titer of the preparation in question. The exact doses and the regimen of administration can be chosen by the physician responsible for the patient, infected by *Helicobacter pylori*. Routine experimentation and later, with increasing experience of this method, empirical information will suffice to establish the required amount. Multiple dosages may be used, as needed, to provide the desired level of therapeutic or prophylactic effect. The immunoglobulin preparations according to the present invention can also, being free from adverse side effects and imposing practically no danger of overdosing, be taken prophylactically or therapeutically by a person without medical supervision.

**[0049]** A therapeutical effect can be attained, except with the specific antibody according to the present invention, also

with at least two Fab-fragments of said antibody. Said embodiment is also encompassed by the scope of the present invention.

**[0050]** According to yet another embodiment, avirulent microorganisms, preferably bacteria, are used as expression systems for the specific antibody according to the present invention. An "avirulent microorganism" in this context is a microorganism which has the ability to colonize and replicate in an infected individual, but which does not cause disease symptoms associated with virulent strains of the same species of microorganism. The definition inherent in the GRAS (Generally Regarded As Safe) concept can be applied here. A GRAS-organism is suitable for use according to the present invention, provided that the organism externalises the antibody or can be modified to this effect. The term "microorganism" as used herein includes bacteria, protozoa and unicellular fungi. Preferably, bacteria are used as expression systems, e.g. bacteria of the genus *Lactobacillus*, *Streptococcus* or *Enterobacteriae*. The above mentioned expression system can be utilised in vitro for the production of the specific antibody according to the present invention or, according to a further embodiment of the invention, the micro-organism constituting the expression system can be administered directly to the patient. The micro-organisms can be harvested and administered as such, but they are preferably mixed with a suitable carrier, mixed in a suitable foodstuff, lyophilised, encapsulated or treated in any other conventional way, used for the delivery of viable microorganisms to the gastrointestinal tract.

**[0051]** According to yet another embodiment, avirulent microorganisms, preferably bacteria, are used as expression systems for the specific adhesin protein according to the present invention. An "avirulent microorganism" in this context is a microorganism which has the ability to colonize and replicate in an infected individual, but which does not cause disease symptoms associated with virulent strains of the same species of microorganism. The definition inherent in the GRAS (Generally Regarded As Safe) concept can be applied here. A GRAS-organism is suitable for use according to the present invention, provided that the organism externalises the adhesin protein or can be modified to this effect. The term "microorganism" as used herein includes bacteria, protozoa and unicellular fungi. Preferably, bacteria are used as expression systems, e.g. bacteria of the genus *Lactobacillus*, *Streptococcus* or *Enterobacteriae*. The above mentioned expression system can be utilised in vitro for the production of the specific adhesin according to the present invention or, according to a further embodiment of the invention, the micro-organism constituting the expression system can be administered directly to the patient. The micro-organisms can be harvested and administered as such, but they are preferably mixed with a suitable carrier, mixed in a suitable foodstuff, lyophilised, encapsulated or treated in any other conventional way, used for the delivery of viable microorganisms to the gastrointestinal tract.

**[0052]** The exact doses and the regimen of administration of said micro-organisms can be chosen by the physician responsible for the patient, infected by *Helicobacter pylori*. Routine experimentation and later, with increasing experience of this method, empirical information will suffice to establish the required amount. Multiple dosages may be used, as needed, to provide the desired level of therapeutic or prophylactic effect. The avirulent micro-organism expressing the antibody or adhesin protein according to the present invention

can also, being free from adverse side effects and imposing practically no danger of overdosing, be taken prophylactically or therapeutically by a person without medical supervision. A preferred carrier in this specific application is a food-stuff, e.g. a fermented product such as fermented cereal or dairy product.

**[0053]** The creation of previously mentioned expression systems and still earlier mentioned methods of creating hybridomas and transgenic animals can include steps involving recombinant DNA techniques. Recombinant DNA techniques are now sufficiently well known and widespread so as to be considered routine. In very general and broad terms, recombinant DNA techniques consist of transferring part of the genetic material of one organism into a second organism, so that the transferred genetic material becomes a permanent part of the genetic material of the organism to which it is transferred. Methods for achieving this are well known and the mere choice of specific methods for achieving the objectives, set out in the present description and claims, fall under the scope of the invention.

**[0054]** It is possible, that *H. pylori* alone or together with related slow-acting bacteria are involved in the genesis and aggravation of other chronic inflammatory diseases in the gastrointestinal tract. It is obvious for a skilled practitioner how to modify the present invention, within the scope of the claims, to gain utility in the treatment and/or prevention of such diseases. Examples of such diseases are ulcerative colitis, Crohn's disease, sarcoidosis, Wegener's granulomatosis and other vasculithic disorders, as well as various neoplasms, including carcinomas of the colon, pancreas and prostate.

#### EXAMPLES

**[0055]** *H. pylori* strain CCUG 17875 was obtained from CCUG, Göteborg, Sweden. Strain A5, a gastric ulcer isolate, from Astra Arcus, Södertälje, Sweden. Strains P466 and MO19 were described previously (Borén et. al, Science, 262, 1892 (1993)). Strain 26695 came from Dr. K. A. Eaton, The Ohio State University and its genome was recently sequenced by TIGR, Rockville, Md., USA. The panel of 45 *H. pylori* clinical isolates came from the University Hospital in Uppsala, Sweden. Bacteria were grown at 37°C. in 10% CO<sub>2</sub> and 5% O<sub>2</sub> for 48 h.

**[0056]** All blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosaccharides to serum albumin were from IsoSep AB, Tullinge, Sweden. The RIA was performed according to Falk et al. (Meth. Enzymol., 236, 353, 1994) with some modifications; the H-1, Le<sup>b</sup>, Le<sup>a</sup>, H-2, Le<sup>x</sup> and Le<sup>y</sup> glycoconjugates were <sup>125</sup>I-labeled by the Chloramine T method. 1 ml of bacteria (A600=OD 0.10) was incubated with 300 ng of <sup>125</sup>I-labelled conjugate (i.e. an excess of receptors) for 30 min. in phosphate buffered saline (PBS), 0.5% albumin, 0.05% Tween-20 (BB-buffer). After centrifugation, <sup>125</sup>I-activity in the bacterial pellet was measured by gamma scintillation counting.

**[0057]** In this study the present inventors' first biochemically characterized and identified the *H. pylori* blood group antigen binding adhesin, BabA. *H. pylori* strains were analysed for binding to soluble <sup>125</sup>I-labeled fucosylated blood group antigens (FIG. 1A). Binding of these strains to the soluble blood group antigens correlate with adherence in situ. The prevalence of blood group antigen binding (BAB)-activity was assessed among 45 clinical *H. pylori* isolates and the majority of the isolates, 71%, express Le<sup>b</sup> antigen binding

properties (data not shown). In contrast, none of the reference strains (FIG. 1A), or strains from the panel of 45 clinical isolates, bind to the Le<sup>a</sup>, H-2, Le<sup>x</sup>, or Le<sup>y</sup> antigens. These results support our previous findings of high receptor specificity for the Le<sup>b</sup> and H-1 blood group antigens and demonstrate the high prevalence of BAB activity among clinical isolates.

**[0058]** Based on the presence or absence of virulence factors such as the Cytotoxin associated gene A (CagA) and the Vacuolating cytotoxin A (VacA), *H. pylori* strains are classified as type I or type II strains. *H. pylori* isolates from patients with duodenal ulcers most often express the VacA and the CagA-proteins, i.e. type-I strains. By definition, type II strains express neither markers. Twenty-one clinical isolates previously defined for expression of CagA and VacA were analysed for Le<sup>b</sup> antigen binding properties. Expression of CagA was found to correlate with bacterial binding to the Le<sup>b</sup> antigen (Table 1). The cagA gene belongs to a 40 kb pathogenicity island that encodes components of secretion and transport systems. These findings could indicate functional crosstalk between the cag pathogenicity island and the BabA adhesin gene, for the correct presentation of the BabA adhesin protein in the bacterial outer membrane.

**[0059]** To further characterize BabA, the present inventors determined the affinity constant (K<sub>a</sub>) between BabA and the Le<sup>b</sup> antigen. Since K<sub>a</sub>-values are based on equilibrium conditions (13), the present inventors first analysed the interaction by performing receptor displacement analysis. *H. pylori* CCUG 17875 (positive for Le<sup>b</sup> binding, FIG. 1A) was first incubated with <sup>125</sup>I-labeled Le<sup>b</sup> glycoconjugate. Then unlabeled Le<sup>b</sup> glycoconjugate was added in a dilution series. The unlabeled Le<sup>b</sup> conjugate displaced the bound <sup>125</sup>I-labeled Le<sup>b</sup> glycoconjugate efficiently (FIG. 1B). The results demonstrate that the receptor-adhesin complex formed is in a true state of equilibrium. An equivalent excess of Le<sup>a</sup> glycoconjugate did not dissociate the Le<sup>b</sup>-BabA complex, verifying the high receptor specificity (FIG. 1B). The K<sub>a</sub>-value for the Le<sup>b</sup>-BabA complex of strain CCUG 17875 was titrated with Le<sup>b</sup> glycoconjugate in concentrations from 10 ng to 260 ng/ml and determined to be of a high affinity close to 1×10<sup>10</sup> M<sup>-1</sup>, (FIG. 1C). The number of Le<sup>b</sup> glycoconjugate molecules bound to BabA on the bacterial cell surface was calculated to be around 500 per cell. This number is similar to the number of fimbriae organelles on the surface of *E. coli* (14). However, for the BabA adhesin, the calculations are based on the assumption that the majority of bacterial cells in the experiment exhibit an equal number of adhesin molecules with Le<sup>b</sup> antigen binding properties.

TABLE 1

BAB activity among <i>H. pylori</i> Type I and Type II strains		
Type	Strain	BAB Activity
Type I CagA <sup>+</sup> , VacA <sup>+</sup>	CCUG 17874	-
	G39	-
	G11	-
	G20	-
	G27	+
	G56	+
	G106	-
	G109	+
	932	+
	Bal85	+
	87A300	+

TABLE 1-continued

BAB activity among <i>H. pylori</i> Type I and Type II strains		
Type	Strain	BAB Activity
Type Ia CagA <sup>+</sup> , VacA <sup>-</sup>	931	+
	Ba99	+
	Ba179	+
	Ba194	+
Type Ib CagA <sup>-</sup> , VacA <sup>+</sup>	G12	-
TypeId	G104	-
AcagA, VacA <sup>+</sup>	Tx30	-
Type II	G21	-
CagA <sup>-</sup> , VacA <sup>-</sup>	G50	-
	G198	-

**[0060]** To determine the prevalence of BabA in the bacterial population, strain CCUG 17875 was incubated with Le<sup>b</sup> or Le<sup>a</sup> antigens, and bacterial binding activity was visualised by confocal fluorescence microscopy (FIG. 2, upper panel). The analyses demonstrate the high prevalence of BabA binding activity in the bacterial population to the Le<sup>b</sup> antigen (FIG. 2A, green staining) and the complete lack of binding to the Le<sup>a</sup> antigen (FIG. 2B, red counter staining).

**[0061]** Next, the localisation and density of BabA on the bacterial cell surfaces was investigated by immunogold electron microscopy. The Le<sup>b</sup> antigen binding activity of the adhesin localised gold particles to the bacterial outer membrane (FIG. 2C). Individual bacterial cells exhibit an equal number of gold particles (data not shown). When the Le<sup>b</sup> antigen was substituted with the Le<sup>a</sup> antigen (lacking receptor activity), no gold particles were detected (FIG. 2D).

**[0062]** The molecular weight of BabA was characterized by receptor overlay analysis. A protein extract of strain CCUG 17875 was separated on SDS-PAGE and blotted to a membrane. The membrane was incubated with biotinylated Le<sup>b</sup> glycoconjugate, followed by detection with streptavidin and enhanced chemiluminescence. The BabA adhesin activity corresponds to a single 74 kDa band (FIG. 3A). The 40 kDa band is presumably endogenous peroxidase activity since it stains independently of the Le<sup>b</sup> conjugate overlay (lane 3). BabA was very heat stable and could regain some activity after heating to 97° C. (FIG. 3A, lane 2). The panel of strains exhibited the same molecular weight of BabA (FIG. 3B).

**[0063]** To purify BabA, a novel technique was developed, Receptor Activity Directed Affinity Tagging (ReTagging). Multi-functional crosslinking agents with radiolabeled donating tags have been previously used for receptor-ligand characterization studies. However, the use of affinity donating tags, such as biotin residues presented on flexible spacer structures, adds a new dimension to the applicability of crosslinker technology. An affinity tag, biotin, is transferred to the adhesin protein by the receptor activity and is used for further identification and for affinity purification of the adhesin part of the interaction, by streptavidin (FIG. 4A, B).

**[0064]** A multi-functional crosslinking agent with a biotin donating handle was attached to the Le<sup>b</sup> glycoconjugate. The receptor activity of the Le<sup>b</sup> glycoconjugate subsequently directed the targeted biotin tagging of the BabA adhesin protein (FIG. 4A, B). After crosslinking, the bacterial protein from strains A5, P466, and CCUG 17875 were separated on SDS-PAGE. Immunodetection with streptavidin demonstrated a biotin tagged protein, with the molecular weight of

74 kDa (FIG. 3C) (28). These results support the estimates of the molecular weight from the previous overlay analyses (FIG. 3B). Strain MO19 devoid of Le<sup>b</sup> antigen binding properties (FIG. 3B) (FIG. 1A), was negative for binding also in this set of analyses (FIG. 3C).

**[0065]** The high specificity in the ReTagging technique provided a method for purification of the adhesin protein. Strains CCUG 17875 and A5, that both express the BabA adhesin (FIG. 1A) were processed by the ReTagging technique using crosslinker labelled Le<sup>b</sup> receptor conjugate as the biotin donor. After crosslinking, bacteria were suspended in SDS sample buffer. Streptavidin coated magnetic beads were subsequently added to the solubilised proteins, and biotin tagged BabA was extracted (FIG. 4C). The N-terminal 20 amino acid sequences of the BabA adhesins from strains CCUG 17875 (Australia) and A5 (Sweden) were found to be identical, indicating a biologically conserved protein (FIG. 5). Recently, a series of outer membrane proteins from *H. pylori* were characterized. These proteins, HopA-E, are homologous in their N-terminal sequences to BabA (17), possible indicating a motif for a common secretion mechanism. The biotin tagged BabA adhesin was purified more than 3000-fold from the cell extract, and the yield was calculated to 20%. However, based on data from the Scatchard plots, the level of available BabA adhesin would be about 5-times higher, i.e. approximately 1 mg adhesin/750 mg bacterial protein, which nevertheless could be the reason for the high signal to noise ratio (FIG. 3B). The purification of BabA via the ReTagging technique indicates the potential of this technique for the purification of lectins in complex receptor-ligand interactions, such as the selectin family of cell adhesion molecules.

**[0066]** To clone the gene encoding BabA, the N-terminal 20 aa sequence was utilised for the construction of degenerate primers (18). Two sets of clones were identified which both encode two different but very similar proteins. Both genes code for proteins having almost identical N-terminal domains and identical C-terminal domains, complicating the identification of the functional BabA gene. (FIG. 5). To identify the corresponding gene, the BabA adhesin was purified in large scale by ReTagging. This provided enough protein for an extended amino terminal sequence. 41 amino acids were identified and these residues unambiguously discriminated between the two genes by the differences in aa-positions 28, 35, 37, 38 and 41 (FIG. 5). The gene encoding BabA was named babA and correspond to a basic protein with a pI of 9.4 and a molecular weight of 78 kDa, i.e. of slightly higher molecular weight than that predicted from the SDS PAGE analyses (FIG. 3). The other gene, babB, corresponds to a protein of a calculated molecular weight of 75.5 kDa. In contrast to babA, the babB gene contains a predicted translational initiation codon (FIG. 5). This could indicate the existence of a third bab gene in the genome or mechanisms for recombination activities. Interestingly, the bab-genes were also detected in strains lacking Lewis b binding properties (data not shown). Gene cassette systems have been shown to promote antigenic variation in *Neisseria gonorrhoeae* (19). Another possibility would be the presence of similar genes coding for adhesins with differences in receptor specificity/host tissue tropism (20). Gene inactivation experiments targeting the bab-genes could aid in understanding this complex gene organisation.

**[0067]** Immunisation experiments with adhesins from *Bordetella pertussis* (21) indicate the potential for outer mem-

brane proteins to act as vaccine candidates (discussed in ref. 22). In a mouse model for persistent *H. pylori* infection, oral immunisation with *H. pylori* antigens proved protective against *H. pylori* infection (10). However, results from animal models are difficult to evaluate for human specific pathogens, such as *H. pylori* and Polio virus. For Polio, an animal model has been achieved by expressing the virus receptor in transgenic mice (23). A similar strategy was taken for *H. pylori*. A transgenic mouse was constructed by the use of an  $\alpha$ 1,3/4-fucosyltransferase, driving the synthesis of the human specific Le<sup>b</sup> antigen in the gastrointestinal tract (24). The Lewis b mouse can be useful for the evaluation of the role of the BabA adhesin as a colonisation/virulence factor and in addition for the evaluation of BabA as a vaccine candidate against acid peptic disease and gastric adenocarcinoma.

**[0068]** In the present study the ReTagging technique was used for the purification of the adhesin part of the microbial receptor-ligand interaction. By the use of purified adhesin/lectin-protein, the ReTagging technique could, in addition, be used to further study the receptor part of the interaction. Identification of the biologically active receptor structure, carrying Le<sup>b</sup> oligosaccharides, would aid in the understanding of the mechanisms supporting the chronic *H. pylori* infection.

**[0069]** Inhibition of *H. pylori* binding to <sup>125</sup>I-labeled Lewis b antigen by preparations is presented graphically, as a function of antibody concentration (mg/ml) in FIG. 6: 1 ml aliquots of *H. pylori* bacteria ( $A_{600}$ =OD 0.10) were pre-incubated with dilution series of antibody preparations, in 0.01-10 mg/ml for 2 hours in phosphate buffered saline (PBS), 0.5% albumin, 0.05% Tween-20. Then 500 ng of <sup>125</sup>I-labeled conjugate (i.e. an excess of receptor structure) was added and incubated for 30 minutes. After centrifugation, <sup>125</sup>I-activity in the bacterial pellet was measured by gamma scintillation counting. The Lewis b blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosaccharides to serum albumin were from IsoSep AB, Tullinge, Sweden.

**[0070]** Western blot detection of the BabA adhesin by the different antibody preparations is presented in FIG. 7: Molecular weight rainbow marker (2  $\mu$ L) from Amersham, Buckinghamshire, England, was dissolved in SDS sample buffer (lane 1). Approx. 100 ng of purified BabA adhesin (approx. 74 kDa with degradation product of approx. 55 kDa) was dissolved in SDS sample buffer (lane 2). SDS solubilized protein extracts of strain CCUG 17875 were prepared by dissolving the bacterial pellet corresponding to 0.15 ml of bacteria ( $A_{600}$ =OD 0.10) by SDS sample buffer (lane 3). The 3 protein samples were then boiled at 100° C. for 5 minutes. The proteins were separated on SDS-PAGE, and transferred to a PVDF-membrane for Western blot immuno analysis. Five sets of PVDF-membranes were prepared. The PVDF membranes were blocked/incubated overnight with 4% human sera/plasma, in phosphate buffered saline, from a patient with no *H. pylori* infection, i.e. with no serum antibodies against *H. pylori*. The membrane was then washed in phosphate buffered saline (PBS), 0.5% albumin, 0.05% Tween-20, followed by the addition of the antibody preparations. The sets of membranes were incubated with the following 5 antibody preparations; 1) pooled human sera from *H. pylori* infected patients, diluted 1:500. 2) Chicken antibodies (positive) 1 mg/ml diluted 1:100 $\times$ . 3) Bovine I preparation of antibodies, 1 mg/ml diluted 1:100 $\times$ . 4) Bovine II preparation of antibodies, 1 mg/ml diluted 1:100 $\times$ . 5) Bovine III prepa-

ration of antibodies, 1 mg/ml diluted 1:100 $\times$  (indicated in the figure). These antibodies were incubated with the membrane for 2 hours followed by extensive washings in phosphate buffered saline (PBS), 0.05% Tween-20, followed by the addition of secondary anti-human, anti-chicken, and anti-bovine antibodies labeled with HRP-peroxidase (from DAKO, Denmark), all diluted 1:2000 $\times$ . Membranes were incubated for 1 hour, followed by extensive washings in phosphate buffered saline (PBS), 0.05% Tween-20. The membranes were developed with enhanced chemoluminescens (ECL) from Amersham. The results show, that the antigenic response against the adhesin is strongly enhanced in the bovine preparations. This finding is also supported by the inhibition data in FIG. 6.

**[0071]** Western blot analyses of *H. pylori* proteins by the different antibody preparations are shown in FIG. 8. 2 clinical isolates (1-2) from Dr. Lars Engstrand, Department of Clinical Microbiology and Cancer epidemiology, University Hospital, Uppsala, Sweden and strain CCUG 17875 (3), from Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden, and strain 52 (4) from Prof. Torkel Wadström, Dept. Medical Microbiology, Lunds University, were prepared for SDS-PAGE electrophoresis. Bacterial pellets corresponding to 0.15 ml of bacteria ( $A_{600}$ =OD 0.10) were dissolved in SDS sample buffer and heated to 100° C. for 5 minutes. The proteins were separated on SDS-PAGE, and transferred to PVDF-membranes for Western blot immuno analysis. The western blot analyses were as described above, i.e. the sets of membranes were incubated with the following 4 antibody preparations; 1) pooled human sera from *H. pylori* infected patients, diluted 1:500. 2) Chicken antibodies (positive) 1 mg/ml diluted 1:100 $\times$ . 3) Bovine I preparation of antibodies, 1 mg/ml diluted 1:100 $\times$ . 4) Bovine III preparation of antibodies, 1 mg/ml diluted 1:100 $\times$  (indicated in the figure). These antibodies were incubated with the membrane for 2 hours followed by extensive washings in phosphate buffered saline (PBS), 0.05% Tween-20, followed by the addition of secondary anti-human, anti-chicken, and anti-bovine antibodies labeled with HRP-peroxidase (from DAKO, Denmark), all diluted 1:2000 $\times$ . Membranes were incubated for 1 hour, followed by extensive washings in phosphate buffered saline (PBS), 0.05% Tween-20. The membranes were developed with enhanced chemoluminescens (ECL) from Amersham. The results show, that the chicken antibodies and the bovine preparations reacts nearly identically against all four strains, indicating conserved properties in strains of different geographical origin.

**[0072]** Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

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- [0080] 8. *H. pylori* strain CCUG 17875 was obtained from CCUG, Göteborg, Sweden. Strain A5, a gastric ulcer isolate, came from Astra Arcus, Södertälje, Sweden. Strains P466 and MO19 were described previously (7). Strain 26695 came from Dr. K. A. Eaton, The Ohio State University, and its genome was recently sequenced by The Institute for Genomic Research (TIGR), Rockville, Md. (J.-F. Tomb, et al, abstract 3B: 059, IX International Workshop on Gastrointestinal Pathology and *Helicobacter pylori*, Copenhagen, Denmark, 1996). The panel of 45 *H. pylori* clinical isolates came from the University Hospital in Uppsala, Sweden. Bacteria were grown at 37°C in 10% CO<sub>2</sub> and 5% O<sub>2</sub> for 48 h.
- [0081] 9. All blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosaccharides to serum albumin (7, 25), were from IsoSep AB, Tullinge, Sweden. The RIA was performed according to ref. 26 with some modifications; The H-1, Le<sup>b</sup>, Le<sup>a</sup>, H-2, Le<sup>x</sup>, and Le<sup>y</sup> glycoconjugates were <sup>125</sup>I-labeled by the Chloramine T method. 1 ml of bacteria (A<sub>600</sub>=OD 0.10) was incubated with 300 ng of <sup>125</sup>I-labeled conjugate (i.e. an excess of receptors) for 30 min. in phosphate buffered saline (PBS), 0.5% albumin, 0.05% Tween-20 (BB-buffer). After centrifugation, <sup>125</sup>I-activity in the bacterial pellet was measured by gamma scintillation counting.
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- [0090] 18. The BabA N-terminal sequence analysis was used to make degenerate oligonucleotides, which were used in PCR to obtain an amplified fragment from the chromosome of the babA gene. A 59 bp fragment was identified and used as probe for the screening of a low-copy plasmide (pACYC184) library of Sau3A partially digested chromosomal DNA from strain CCUG 17875.
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- [0099] 27. Cell extracts were prepared in SDS sample buffer without mercapto ethanol and heated at 37° C. or 97° C. for 10 min. before separation on SDS-PAGE. Proteins were blotted onto a PVDF membrane. The membrane was incubated with 1 µg/ml biotinylated Le<sup>b</sup> glycoconjugate or biotinylated albumin (negative control) overnight, labelled as described in ref. 7. After washing in PBS/0.05% Tween-20, the biotinylated structures bound by the BabA band were probed by HRP-streptavidin and detected using ECL reagents (Amersham, Buckinghamshire, England).
- [0100] 28. The bacterial suspension was incubated with Le<sup>b</sup> glycoconjugate, to which the Sulfo-SBED crosslinker (Pierce, Rockville, Ill.) had been conjugated by the N-hydroxysuccinimide ester (NHS), according to the manufacturers specifications. The aryl azide crosslinker group was activated by UV irradiation (360 nm). Bacteria were washed with PBS pH 7.6, 0.05% Tween-20 and protease inhibitors (EDTA and benzamidine) under reducing conditions with 50 mM dithiothreitol (DTT). Bacterial proteins were separated on SDS-PAGE, and the biotin tagged BabA protein was detected by immunodetection (PVDF membrane/HRP-streptavidin and ECL) (FIG. 3C).
- [0101] 29. Strains CCUG 17875 and A5 were first processed by crosslinking and DTT treatment, as above (28), followed by solubilisation in SDS sample buffer. The biotin tagged BabA protein was then extracted with streptavidin coated magnetic beads (Advanced Magnetics Inc., Cambridge, Mass.). The beads were boiled in SDS sample buffer, and bound proteins were eluted and alkylated. The protein preparation was further fractionated by preparative SDS-PAGE (Prep-Cell 491, BioRad, Hercules, Calif.). Fractions with the biotin tagged protein, i.e. the BabA fractions, were identified by immunodetection using streptavidin/ECL. The pooled BabA preparation was then separated on SDS-PAGE and transferred to PVDF membrane. The BabA band was excised and the BabA protein was N-terminally sequenced using a Procise™ 494 instrument (Applied Biosystems, Foster City, Calif.).

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 9

<210> SEQ ID NO 1

<211> LENGTH: 3340

<212> TYPE: DNA

<213> ORGANISM: *Helicobacter pylori*

<400> SEQUENCE: 1

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&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 2781

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Helicobacter pylori

&lt;400&gt; SEQUENCE: 2

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&lt;210&gt; SEQ ID NO 3

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<211> LENGTH: 744
<212> TYPE: PRT
<213> ORGANISM: Helicobacter pylori
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (24)..(64)
<223> OTHER INFORMATION: N-terminal domain of the BabA adhesin

<400> SEQUENCE: 3

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 20          25          30

Gly Tyr Gln Ile Gly Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly
 35          40          45

Ile Gln Asp Leu Ser Asp Asn Tyr Glu Asn Leu Ser Lys Leu Leu Thr
 50          55          60

Arg Tyr Ser Thr Leu Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser
 65          70          75          80

Ala Ile Asn Ala Ala Arg Glu Asn Leu Gly Ala Ser Ala Lys Asn Leu
 85          90          95

Ile Gly Asp Thr Lys Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala
100          105          110

Ile Asn Ala Ala Val Gly Phe Trp Asn Val Leu Gly Tyr Ala Thr Gln
115          120          125

Cys Gly Gly Asn Ala Asn Gly Gln Glu Ser Thr Ser Ser Thr Thr Ile
130          135          140

Phe Asn Asn Glu Pro Gly Tyr Arg Ser Thr Ser Ile Thr Cys Ser Leu
145          150          155          160

Asn Arg Tyr Lys Pro Gly Tyr Tyr Gly Pro Met Ser Ile Glu Asn Phe
165          170          175

Lys Lys Leu Asn Glu Ala Tyr Gln Ile Leu Gln Thr Ala Leu Asn Lys
180          185          190

Gly Leu Pro Ala Leu Lys Glu Asn Asn Gly Thr Val Ser Val Thr Tyr
195          200          205

Thr Tyr Thr Cys Ser Gly Glu Gly Asn Asp Asn Cys Ser Lys Lys Ala
210          215          220

Thr Gly Val Ser Asp Gln Asn Gly Gly Thr Lys Thr Lys Thr Gln Thr
225          230          235          240

Ile Asp Gly Lys Thr Val Thr Thr Thr Ile Ser Ser Lys Val Val Asp
245          250          255

Ser Gln Ala Lys Gly Asn Thr Thr Arg Val Ser Tyr Thr Glu Ile Thr
260          265          270

Asn Lys Leu Asp Gly Val Pro Asp Ser Ala Gln Ala Leu Leu Ala Gln
275          280          285

Ala Ser Thr Leu Ile Asn Thr Ile Asn Thr Ala Cys Pro Tyr Phe Ser
290          295          300

Val Thr Asn Lys Ser Gly Gly Pro Gln Met Glu Pro Thr Arg Gly Lys
305          310          315          320

Leu Cys Gly Phe Thr Glu Glu Ile Ser Ala Ile Gln Lys Met Ile Thr
325          330          335

Asp Ala Gln Glu Leu Val Asn Gln Thr Ser Val Ile Asn Glu His Glu
340          345          350

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 Ala Lys Met Leu Asn Leu Ala His Gln Val Gly Gln Thr Ile Asn Pro  
 385 390 395 400  
 Asp Asn Leu Thr Gly Thr Phe Lys Asn Phe Val Thr Gly Phe Leu Ala  
 405 410 415  
 Thr Cys Asn Asn Lys Ser Thr Ala Gly Thr Ser Gly Thr Gln Gly Ser  
 420 425 430  
 Pro Pro Gly Thr Val Thr Thr Gln Thr Phe Ala Ser Gly Cys Ala Tyr  
 435 440 445  
 Val Glu Gln Thr Ile Thr Asn Leu Asn Asn Ser Ile Ala His Phe Gly  
 450 455 460  
 Thr Gln Glu Gln Gln Ile Gln Gln Ala Glu Asn Ile Ala Asp Thr Leu  
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 Val Asn Phe Lys Ser Arg Tyr Ser Glu Leu Gly Asn Thr Tyr Asn Ser  
 485 490 495  
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 Val Val Gly Lys Lys Asn Asn Pro Tyr Ser Pro Gln Gly Ile Glu Thr  
 515 520 525  
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 Gln Thr Asn Asn Gly Ala Met Asn Gly Ile Gly Ile Gln Val Gly Tyr  
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 Phe Phe Asp Tyr Asn His Ala Phe Ile Lys Ser Ser Phe Phe Asn Ser  
 595 600 605  
 Ala Ser Asp Val Trp Thr Tyr Gly Phe Gly Ala Asp Ala Leu Tyr Asn  
 610 615 620  
 Phe Ile Asn Asp Lys Ala Thr Asn Phe Leu Gly Lys Asn Asn Lys Leu  
 625 630 635 640  
 Ser Val Gly Leu Phe Gly Gly Ile Ala Leu Ala Gly Thr Ser Trp Leu  
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 Asn Ser Glu Tyr Val Asn Leu Ala Thr Met Asn Asn Val Tyr Asn Ala  
 660 665 670  
 Lys Met Asn Val Ala Asn Phe Gln Phe Leu Phe Asn Met Gly Val Arg  
 675 680 685  
 Met Asn Leu Ala Arg Ser Lys Lys Lys Gly Ser Asp His Ala Ala Gln  
 690 695 700  
 His Gly Ile Glu Leu Gly Leu Lys Ile Pro Thr Ile Asn Thr Asn Tyr  
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 Tyr Leu Asn Tyr Val Phe Ala Tyr  
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<210> SEQ ID NO 4  
 <211> LENGTH: 707  
 <212> TYPE: PRT  
 <213> ORGANISM: Helicobacter pylori  
 <220> FEATURE:  
 <221> NAME/KEY: PEPTIDE  
 <222> LOCATION: (46)..(59)  
 <223> OTHER INFORMATION: Corresponding to the N-terminal domain of the BabA adhesin

<400> SEQUENCE: 4

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His Ala Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly
          20           25           30

Glu Ala Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Gln Leu Ser
          35           40           45

Asp Asn Tyr Glu Lys Leu Asn Asn Leu Leu Asn Asn Tyr Ser Thr Leu
          50           55           60

Asn Thr Leu Ile Lys Leu Ser Ala Asp Pro Ser Ala Ile Asn Asp Ala
          65           70           75           80

Arg Asp Asn Leu Gly Ser Ser Ala Lys Asn Leu Leu Asp Val Lys Thr
          85           90           95

Asn Ser Pro Ala Tyr Gln Ala Val Leu Leu Ala Leu Asn Ala Ala Val
          100          105          110

Gly Leu Trp Gln Val Thr Ser Tyr Ala Phe Thr Ala Cys Gly Pro Gly
          115          120          125

Ser Asn Glu Ser Ala Asn Gly Gly Ile Gln Thr Phe Asn Asn Val Pro
          130          135          140

Gly Gln Lys Thr Thr Thr Ile Thr Cys Asn Ser Tyr Tyr Gln Pro Gly
          145          150          155          160

His Gly Gly Pro Ile Ser Thr Ala Asn Tyr Ala Lys Ile Asn Gln Ala
          165          170          175

Tyr Gln Ile Ile Gln Lys Ala Leu Thr Ala Asn Glu Ala Asn Gly Asp
          180          185          190

Gly Val Pro Val Leu Ser Asp Thr Thr Thr Lys Leu Asp Phe Thr Ile
          195          200          205

Gln Gly Asp Lys Arg Thr Gly Gly Arg Pro Asn Thr Pro Lys Lys Phe
          210          215          220

Pro Trp Ser Asp Gly Lys Tyr Ile His Thr Gln Trp Ile Asp Thr Thr
          225          230          235          240

Pro Gln Ser Thr Glu Thr Lys Ile Asn Thr Glu Asn Asn Ala Gln Glu
          245          250          255

Leu Leu Lys Gln Ala Ser Ile Ile Ile Thr Thr Leu Asn Glu Ala Cys
          260          265          270

Pro Asn Phe Gln Asn Gly Gly Ser Gly Tyr Trp Gln Gly Ile Ser Gly
          275          280          285

Asn Gly Thr Met Cys Gly Met Phe Lys Asn Glu Ile Ser Ala Ile Gln
          290          295          300

Gly Met Ile Ala Asn Ala Gln Glu Ala Val Ala Gln Ser Lys Ile Val
          305          310          315          320

Ser Glu Asn Ala Gln Asn Gln Asn Asn Leu Asp Thr Gly Lys Pro Phe
          325          330          335

Asn Pro Phe Thr Asp Ala Ser Phe Ala Gln Ser Met Leu Lys Asn Ala
  
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&lt;400&gt; SEQUENCE: 5

Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly Glu Ala  
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 Ala Gln Met Val  
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&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 60

&lt;212&gt; TYPE: DNA

<213> ORGANISM: *Helicobacter pylori*

&lt;400&gt; SEQUENCE: 6

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&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 41

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Helicobacter pylori*

&lt;400&gt; SEQUENCE: 7

Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly Glu Ala  
 1 5 10 15  
 Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Asp Leu Ser Asp Asn  
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 Tyr Glu Asn Leu Ser Lys Leu Leu Thr  
 35 40

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 20

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Helicobacter pylori*

&lt;400&gt; SEQUENCE: 8

Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly Glu Ala  
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 Ala Gln Met Val  
 20

&lt;210&gt; SEQ ID NO 9

&lt;211&gt; LENGTH: 41

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Helicobacter pylori*

&lt;400&gt; SEQUENCE: 9

Glu Asp Asp Gly Phe Tyr Thr Ser Val Gly Tyr Gln Ile Gly Glu Ala  
 1 5 10 15  
 Ala Gln Met Val Thr Asn Thr Lys Gly Ile Gln Gln Leu Ser Asp Asn  
 20 25 30  
 Tyr Glu Lys Leu Asn Asn Leu Leu Asn  
 35 40

What is claimed is:

1. A vaccine comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:6.

2. A test kit comprising a monospecific antisera that recognizes a BabA antigen and comprises an immunoglobulin

that binds said BabA antigen via a variable region, wherein said Bab A adhesin protein is produced using an isolated and purified bacterial blood group antigen binding protein (BabA) from *Helicobacter pylori* species, wherein said BabA protein binds specifically to fucosylated Lewis<sup>b</sup> type I and H-1 blood group antigen-glycoconjugates and,

- wherein said BabA protein contains less than 20% bacterial protein impurities, has a molecular weight in the interval of 73 to 75 kDa as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and is not a HopA, HopB, HopC, HopD, or HopE protein.
3. An isolated monospecific immunoglobulin composition from sera which recognizes a BabA antigen and binds said BabA antigen via a variable region and exhibits specific activity to a BabA adhesion protein from *Helicobacter pylori*, wherein said adhesion protein binds Lewis<sup>b</sup> and H-1 blood group antigen-glycoconjugates and is not a HopA, HopB, HopC, HopD or HopE protein.
4. The immunoglobulin composition according to claim 3, wherein said BabA adhesin protein has a molecular weight in the interval of about 70 to 77 kDa as determined by SDS-PAGE.
5. The immunoglobulin composition according to any one of claims 3 or 4 wherein said BabA adhesin protein comprises the following amino acid sequence: EDDGFYTSVGY-QIGEEAAQMV (SEQ ID NO:5) or homologues thereof.
6. An isolated monospecific antibody, which recognizes a BabA antigen and binds said BabA antigen via a variable region and exhibits specific activity to a BabA adhesion protein from *Helicobacter pylori* that binds Lewis<sup>b</sup> and H-1 blood group antigen-glycoconjugates and is not a HopA, HopB, HopC, HopD or HopE protein.
7. The antibody according to claim 6, wherein said BabA adhesin protein has a molecular weight in the interval of about 70 to 77 kDa as determined by SDS-PAGE.
8. The antibody according to claim 6, wherein said BabA adhesin protein comprises the following amino acid sequence: EDDGFYTSVGY-QIGEEAAQMV (SEQ ID NO:5) or homologues thereof.
9. The antibody according to claim 6, wherein said antibody is a monoclonal antibody.
10. A method of manufacturing an immunoglobulin composition according to claim 3, comprising the following steps: immunizing an animal with Lewis<sup>b</sup> binding protein or fractions thereof, expressed by *Helicobacter pylori*, isolating the immunoglobulin fraction from an excretion of said host animal, and purifying the immunoglobulin preparation to obtain an isolated monospecific immunoglobulin composition.
11. The method according to claim 10, wherein said animal is a cow and the immunoglobulin fraction is isolated from the milk, preferably the colostrum thereof.
12. The method according to claim 10, wherein said animal is a chicken and the immunoglobulin fraction is isolated from the egg yolk thereof.
13. A method of manufacturing an antibody according to claim 6, wherein the method comprises the following steps: immunizing an animal with a Lewis<sup>b</sup> binding protein (BabA) or fractions thereof, expressed by *Helicobacter pylori*, fusing immunised, immunoglobulin producing cells with a neoplastic cell line, selecting and growing cells expressing said antibody, and purifying the antibodies.
14. The method of claim 13, further comprising expressing said antibody by a culture of viable microorganisms in an expression system, where said microorganism or organisms are generally recognized as safe (GRAS) and genetically modified to express said antibody.
15. The method according to claim 14, wherein said microorganism is selected from the group consisting of bacteria of the species *Lactobacillus*, *Staphylococcus* and *Enterobacteriaceae*.
16. A pharmaceutical preparation for treating an individual having or identified as being at risk of developing *Helicobacter pylori* infection, gastric ulcers or acid peptic disease in humans comprising the immunoglobulin composition according to claim 3.
17. A method of treating an individual having or identified as being at risk of developing gastric ulcers comprising administering to a subject in need thereof the immunoglobulin composition according to claim 3.
18. A method of treating an individual having or identified as being at risk of developing acid peptic disease comprising administering to a subject in need thereof the immunoglobulin composition according to claim 3.
19. A pharmaceutical product for treating an individual having or being identified at risk of developing *Helicobacter pylori* infections, gastric ulcers or acid peptic disease in humans, comprising the antibody according to claim 6.
20. A pharmaceutical product for treating an individual having or being identified at risk of developing gastric ulcers comprising the antibody according to claim 6.
21. A pharmaceutical product for the treating an individual having or being identified at risk of developing peptic disease comprising the antibody according to claim 6.
22. The method according to claim 18, wherein said subject is human and said immunoglobulin composition is administered orally.
23. A method for treating a human having or being identified at risk of developing *Helicobacter pylori* infections, comprising orally administering an effective amount of an antibody according to claim 6 to said human.
24. A method of treating a human having or being identified at risk of developing *Helicobacter pylori* infections, said method comprising orally administering an effective amount of a culture of viable microorganisms in an expression system, wherein said microorganism or organisms are generally recognized as safe (GRAS) and genetically modified to express an antibody according to claim 8 or 9.
25. An expression system comprising a culture of viable microorganisms wherein said microorganism or organisms are generally recognized as safe (GRAS) and genetically modified to express an antibody according to claim 8 or 9.
26. A method for treating a human having or being identified at risk of developing *Helicobacter pylori* infections, said method comprising orally administering an effective amount of a culture of viable microorganisms in an expression system, wherein said microorganisms or organisms are generally recognized as safe (GRAS) and genetically modified to express an adhesion protein according to claim 1.
27. The composition according to claim 4, wherein the molecular weight of said BabA adhesin protein is in the interval of 73 to 75 kDa.
28. The composition according to claim 4, wherein the molecular weight of said BabA adhesin protein is about 73.5 kDa.
29. The antibody according to claim 6, wherein the molecular weight of said BabA adhesin protein is in the interval of 73 to 75 kDa.
30. The antibody according to claim 4, wherein the molecular weight of said BabA adhesin protein is about 73.5 kDa.

\* \* \* \* \*

专利名称(译)	幽门螺杆菌粘附素结合基因抗原		
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#### 摘要(译)

分离并纯化新型幽门螺杆菌血型抗原结合 (BAB) 粘附素蛋白, 其中所述蛋白质或其部分特异性结合岩藻糖基化血型抗原。在本申请中公开了所述粘附素的蛋白质序列。同时公开了产生高度相似蛋白质的两种基因babA和babB的DNA序列。所述粘附素和/或DNA可用于诊断和治疗和/或预防针对幽门螺杆菌诱导的感染, 例如, 胃炎和酸性消化性疾病, 即主动接种疫苗。一种新的免疫球蛋白组合物, 其对结合幽门螺杆菌粘附素的Lewisb抗原表现出特异性活性, 或优选地, 对所述粘附素的单克隆和/或多克隆抗体提供了一种新的和更有效的治疗和/或预防由幽门螺杆菌引起的胃肠疾病的方法。幽门螺杆菌或其他螺杆菌属物种, 即被动疫苗接种。

