BACTERIAL PATHOGENICITY

Identification of heparan sulphate binding surface proteins of *Helicobacter pylori*: inhibition of heparan sulphate binding with sulphated carbohydrate polymers

M. UTT*† and T. WADSTRÖM*

*University of Lund, Institute of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden and †Bional Ltd, Riia 185, EE2400 Tartu, Estonia

Heparan sulphate binding to cells of the gastric pathogen *Helicobacter pylori* at pH 4–6 is common. Binding was inhibited by various unlabelled sulphated polysaccharides and at high ionic strength and pH, but not by carboxylated or non-sulphated compounds. The inhibition by various sulphated compounds such as dextran sulphate and carrageenans was related to the sulphate content and not to the carbohydrate polymer backbone. The IC₅₀ values for heparin and dextran sulphate for *H. pylori* strain 25 were calculated as 3.55×10^{-7} M and 5.01×10^{-6} M respectively. Heparin-binding proteins of *H. pylori* are exposed on the cell surface, as shown by biotinylation of cell-surface proteins before separation of outer membranes and by an indirect immunofluorescence assay. The strongest biotin-heparin binding by *H. pylori* was observed with a polypeptide in the 55–60 kDa region.

Introduction

Heparan sulphate and other sulphated glycosaminoglycans (GAGs) are exposed on eukaryotic cell surfaces and extracellular matrix (ECM) [1]. Several pathogenic micro-organisms and viruses – such as *Bordetella pertussis*, *Streptococcus mutans*, *Leishmania donovani*, *Trypanosoma cruzi*, *Plasmodium circumsporozoites*, *Chlamydia trachomatis*, human immunodeficiency virus, herpes virus and cytomegalovirus – interact with host cells by binding to sulphated GAG-molecules on cell surfaces and ECM [2–15]. Heparin and heparan sulphate binding to *Staphylococcus aureus* and *Streptococci* of groups A, C, and G with moderate affinity ($K_d = 10^{-5}$ – 10^{-7} M) [16] and to *Helicobacter pylori* with high affinity ($K_d = 10^{-9}$ – 10^{-10} M) [17], equivalent to the high affinity reported for heparin binding to *C. trachomatis* [18], has been reported previously.

Moreover, binding of ¹²⁵I-labelled heparan sulphate to *H. pylori* was shown to be pH and salt dependent [17]. A recent report demonstrated that *H. pylori*

heparin-binding proteins (HepBP) are located on the outer-membrane surface of the cells [19].

Materials and methods

Chemicals

Bovine lung heparan sulphate (HS-3, medium content of sulphate groups) was a generous gift from L.-Å. Fransson (Department of Medical Chemistry, Lund University).

Heparin (porcine intestinal mucosa, mol. wt 6000–8000) was purchased from Fluka (Heidelberg, Germany); dextran sulphate (mol. wt 5000 = DS 5000, mol. wt 8000 = DS 8000 and mol. wt 500 000 = DS 500 000), chondroitin sulphate A, B and C, (Ch.SO4 A, Ch.SO4 B and Ch.SO4 C, respectively), fucoidan, carrageenan- λ from *Gigantia aciculaire* and *G. pistillata*, hyaluronic acid from bovine trachea, pentosan polysulphate (PP-SO4), N-acetylneuraminic acid type VIII from sheep submaxillary glands, 2-desoxyglucosamine-2-sulphate, N-acetylglucosamine-6-sulphate, and N-acetylgalactosamine-6-sulphate were purchased from Sigma; bovine submaxillary gland mucin was purchased from Worthington (Freehold, NJ, USA), and dextran (mol. wt 250 000) from Pharmacia (Uppsala, Sweden). Dextran sulphate mol. wt 5000 (DS 5000), dextran sulphate mol. wt 500 000 (DS

Received 27 Oct. 1995; revised version accepted 18 March 1996.

Corresponding author: Professor T. Wadström.

Publication was delayed from Oct. 1996 at the request of the authors.

500,000), and long-arm biotin hydrazide and amino-carbazole were purchased from Sigma; basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) were purchased from Boehringer Mannheim (Mannheim, Germany). Blocking (SAT-1) and washing buffer were obtained from M. Rucheton, Orston Laboratoire, Montpellier, France. All chemicals were of analytical grade and Millipore grade de-ionised water was used in all experiments.

Strains and culture conditions

Seven strains of *Helicobacter pylori* (25, 1139, 17874, 17875, 915, 66 and 33) from the University of Lund collection were studied.

All strains were grown in micro-aerophilic conditions at 37°C for 3 days on GAB-CAMP agar with defibrinated horse blood 5% v/v [20]. The bacteria were harvested in 0.01 M sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl (PBS), washed once with PBS and suspended in PBS, pH 7.2 or 4.5, to a final density of 10^{10} cfu/ml. Cells were pretreated with water, 1 M LiCl and 0.2 M acid glycine, pH 2.2, at room temperature for 1 h. Cells were harvested by centrifugation at 3000 g for 15 min and suspended in PBS, pH 7.2 or 4.5, to a final cell density of 10^{10} cfu/ml.

Binding assay

Heparan sulphate (HS-3) was derivatised at the α -amino group of the residual serine with *p*-hydroxy-phenyl propionate and labelled with 125 I as described by Fransson *et al.* [21]. The binding of 125 I-labelled HS-3 to *H. pylori* was quantified as described previously [16]. Briefly, samples of 125 I-labelled HS-3 (c. 25 000 cpm) were added to cell suspensions (10^9 cfu in 200 μ l) in a selected binding buffer (i.e., buffers with different pH value or different salt content) supplemented with bovine serum albumin 0.1% and 0.15 M NaCl and kept at 20°C for 1 h. To each cell suspension, 2 ml of a binding buffer (PBS, pH 7.2 or 4.5, presence or absence of NaCl) containing Tween 20 0.05% was added. The tubes were centrifuged (2000 g 10 min), supernates were aspirated and bound radioactivity was determined in a gamma-counter (Clini-Gamma, LKB-Pharmacia, Uppsala, Sweden). All experiments were performed in duplicate and the mean value of the bound HS-3 was calculated by dividing bound radioactivity by total added radioactivity and correcting for any background radioactivity.

Binding inhibition assay

H. pylori cells (10^9 cfu) in 100 μ l of binding buffer were mixed with 100 μ l of buffer solution containing 250–2.5 μ g putative inhibitor and kept at 20°C for 1 h. 125 I-labelled HS-3 was added to each tube and incubated at 20°C for 1 h. These solutions were centrifuged after washing with incubation buffer and

the residual cell-bound radioactivity in the pellets was determined in the gamma counter.

Preparation of outer membranes

H. pylori outer membranes were prepared as described by Doig and Trust [22]. Briefly, cells were washed with PBS and suspended in PBS containing 1 mM phenyl-methylsulphonyl fluoride (PMSF) as protease inhibitor. Cells were disrupted by sonication (10 $\times$ 30 s at 30 W on ice). Whole cells and cell debris were removed by centrifugation (5000 g, 15 min). Membranes (as cell envelopes) were collected by centrifugation (40 000 g, 1 h) at 4°C. Pellets were collected and treated with sodium lauroylsarcosine 2% for 30 min at room temperature to remove inner membranes. An outer-membrane (OM) preparation was collected by centrifugation (40 000 g, 1 h, 4°C) and suspended in water for SDS-PAGE and Western blotting.

SDS-PAGE and Western blotting

H. pylori OM proteins were separated and analysed by SDS-PAGE (12% gel or gradient gel 5–20%) with the buffer system of Laemmli [23]. Outer membranes, treated with lauroylsarcosine 2%, were washed once with PBS and dissolved in SDS-PAGE sample buffer. After SDS-PAGE, proteins were transferred to a 0.45- μ m nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) or PVDF membrane (Millipore, Bedford, USA) by a semi-dry blotting technique (Ancos, Vig, Denmark). Membranes were blocked with SAT 1 for 15 min at room temperature, cut into 5–10-mm strips and used in a biotin-heparin assay.

Biotinylation of heparin

Heparin (2 mg) was dissolved in 500 μ l of 0.1 M sodium acetate buffer, pH 5.5, and 5 $\times$ 20- μ l volumes of periodate solution (20 mg in 850 μ l of water) were added to the heparin solution at 2-min intervals. After incubation for an additional 20 min in the dark, excess periodate was neutralised by adding 10 μ l of sodium metabisulphite solution (19 mg in 100 μ l of water) and 20 μ l of long-arm biotin-hydrazide (1 mg/ml, dissolved in dimethylsulphoxide, DMSO) was added to the heparin solution. After overnight incubation with shaking, 100 μ l of ethanolamine was added and incubated for 1 h. The non-reacted biotin-hydrazide was separated on a Fast Desalt column (Pharmacia, Sweden) equilibrated with water and fractions eluting at the void volume were lyophilised. Biotin-heparin at the concentration 1 mg/ml was dissolved in water and used as a stock solution for the biotin-heparin assay.

Biotin-heparin assay

The strips of nitrocellulose were incubated in 7 ml of binding buffer (for pH study buffers with different pH; or different concentrations of salt, in the presence or

absence of inhibitors) with 1 μg of biotin-heparin (1 μl of aqueous solution, 1 mg/ml) for 4 h or overnight at room temperature with shaking. After washing four times for 7 min with binding buffer containing Tween 20 0.05%, the streptavidin-HRP conjugate (Boehringer Mannheim) at a dilution of 1 in 10 000 was added and incubated for another 1 h at room temperature with shaking. The strips were washed as before and developed with amino carbazole (1 ml) in 20 ml of 50 mM acetate buffer, pH 5.0, containing 20 μl of peroxide.

Binding assay with biotin-labelled growth factors

Binding assays with biotin-labelled bFGF and EGF were performed in PBS at pH 5.5 and 6.5 in the presence or absence of heparin, respectively. The strips were incubated with 50 ng of growth factors. All other conditions were the same as in the biotin-heparin assay.

Results

Binding of HS-3 to *H. pylori* cells of seven of the strains at neutral pH was very low. Only cells of strains 25 and 1139 bound HS-3 to a level above the background at neutral pH. However, HS-3 binding increased dramatically at pH 4.5 up to 60–70% of the total HS-3 for some strains (Fig. 1). The influence of water, 1 M LiCl and acid glycine, pH 2.2, on the binding to *H. pylori* strains 915, 66, 17874 of HS-3 is shown in Fig. 2. Methods commonly used for releasing loosely bound surface proteins from *H. pylori* cells (i.e., acid glycine, chaotropic LiCl solution and water washes), did not affect the binding of ^{125}I -labelled HS-3; on the contrary, an increase in binding was observed at pH 4.5.

HS-3 binding to *H. pylori* 1139 cells at pH 4.5 was strongly inhibited by various sulphated compounds at

Fig. 1. Heparan sulphate (HS-3) binding to strains of *H. pylori* in PBS at pH 4.5.

Fig. 2. Influence of different treatments on heparan sulphate (HS) binding to strains of *H. pylori* in PBS at pH 4.5; ▨, PBS, pH 7.2; □, water; ▤, glycine, pH 2.2; ■, 1 M LiCl.

concentrations of 250 $\mu\text{g}/\text{ml}$ and an increased salt concentration. To differentiate between the ability of compounds to give complete inhibition of binding, the effect of dilutions of various inhibitors was studied (Fig. 3).

More detailed analysis of the binding of ^{125}I -labelled HS-3, heparin and DS 5000 were performed for strains 25 and 17874 (Fig. 4). From these data the IC₅₀ values (i.e., concentration of inhibitor giving

Fig. 3. Percentage inhibition of heparan sulphate binding to *H. pylori* strain 1139 in PBS at pH 4.5 by various inhibitors at different concentrations. —□—, heparin; ...◇..., pp-SO₄; ---○---, Ch.SO₄ A; ---△---, Ch.SO₄ B; ▢—, Ch.SO₄ C; ---◊---, fucoidan; ---▽---, DS 500 000.

Fig. 4. Inhibition of heparan sulphate binding to cells of *H. pylori* strains 25 and 17874 by heparin and DS 5000 in PBS at pH 4.5. —□—, *H. pylori* 17874 + heparin; ...◇..., *H. pylori* 25 + heparin; ---○---, *H. pylori* 17874 + DS 5000; ---△---, *H. pylori* 25 + DS 5000.

50% inhibition) for heparin and DS 5000 were calculated for *H. pylori* strain 25 as 3.55×10^{-7} M and 1.33×10^{-6} M, respectively, and for strain 17874 as 4.73×10^{-7} M and 5.01×10^{-6} M, respectively. The difference in the heparin IC₅₀ value for *H. pylori* strains 25 and 17874 was insignificant, (showing no significant difference in binding properties between these strains); the DS 5000 IC₅₀ value for these two strains differed by five-fold.

OM proteins were separated on SDS-PAGE and analysed by Western blotting with the biotin-heparin assay; three-to-four bands in the range 15–60 kDa were separated, confirming the location of HepBP-s on the cell surface (Fig. 5). Also, biotinylation of cell-surface proteins before separation of OM revealed the same bands as in the biotin-heparin assay (data not shown). Finally, an indirect immunofluorescence assay with a rabbit antiserum raised against a 57-kDa polypeptide in the 55–60 kDa region confirmed the location of this protein on the cell surface (data not shown).

When the pH was increased from 4.5 to 7.5, the binding of labelled heparin decreased and, finally, only the 55–60-kDa polypeptide gave a positive reaction at pH 6.5 and 7.2 in PBS (Fig. 5: lanes 1, 3, 5, 7 and 10). The same polypeptide reacted with biotin-bFGF at pH 5.5 in the presence of heparin, but not in its absence (Fig. 5: lanes 14 and 12). The binding assay with biotin-EGF at pH 6.5 did not reveal any reactive bands with or without heparin (data not shown).

Discussion

A number of studies have shown that heparin, heparan sulphate (HS) and other glycosaminoglycans (GAGs) are involved in the binding of some specific viruses and pathogenic microbes to eukaryotic cells [2–13].

HS binding to *H. pylori* cells at a pH 4–6 seems to be a common feature (Fig. 1). In the upper gastric mucin layer, the pH value is *c.* 2–3, which increases closer to the epithelial cell surface (about pH 7).

1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 5. Biotin-heparin assay with *H. pylori* strain 25 outer-membrane proteins separated on SDS-PAGE, transferred to nitrocellulose and probed with biotin-labelled heparin at pH 4.5–7.5. Lane 1, 50 mM KH₂PO₄, pH 4.5; 3, 50 mM KH₂PO₄, pH 5.5; 5, 50 mM KH₂PO₄, pH 6.5; 7, 50 mM KH₂PO₄, pH 7.5; 10, PBS, pH 7.2; 12, 50 mM KH₂PO₄, pH 5.5, + bFGF; 13, 50 mM KH₂PO₄, pH 6.5, + bFGF; 14, 50 mM KH₂PO₄, pH 5.5, + heparin + bFGF; 2, 4, 6, 8, 9, 11, control with streptavidin-HRP.

Because of high epithelial cell turnover, the mucus layer probably contains cell debris with HS and other cell-surface glycoconjugates, as well as soluble compounds to which *H. pylori* cells may bind. Interestingly, *H. pylori* OM proteins, pre-incubated with heparin, can bind heparin-dependent growth factors and interfere with peptic ulcer healing. The binding of sulphated compounds by *H. pylori* cells may have a significant role in colonisation and HS binding was shown to be inhibited by highly sulphated carbohydrate polymers (Fig. 3).

HS, dermatan sulphate and chondroitin sulphate are glycosaminoglycans on cell surfaces and in the extracellular matrix [24]. The binding of ^{125}I -labelled HS-3 was strong at physiological salt concentrations (at pH 4.5) and was almost abolished if 0.5 M NaCl was added, indicating that mainly ionic interactions are involved. Structurally different polysaccharides gave strong inhibition of ^{125}I -labelled HS-3 binding to *H. pylori* cells, indicating that the oligosaccharide structures are not so important as the density of sulphate groups on these polymers. Interestingly, non-sulphated hyaluronic acid with a similar structure to heparin, HS and chondroitin 4- and 6-sulphates did not inhibit HS binding to cells of the seven *H. pylori* strains, whereas a structurally different heparinoid (pentosan polysulphate) gave a strong inhibition (Fig. 3). On the contrary, sulphated monosaccharide had low inhibitory activity (data not shown).

Monophasic inhibition curves for heparin are typical for non-selective competitors and the IC₅₀ value demonstrates moderate binding affinity of heparin to the HS cell-surface proteins of *H. pylori*.

A number of tissue-invasive bacterial pathogens bind to specific cell-surface glycoproteins and glycolipids on epithelial cells. Following epithelial damage, some of these pathogens may also bind to subepithelial structures such as various extracellular matrix (ECM) molecules to promote tissue colonisation and survival.

Previously studies have shown that *H. pylori* specifically interacts with two major basal membrane ECM molecules, collagen type IV and laminin [25, 26]. The following hypothesis attempts to define the possible role of HS binding to specific surface molecules of *H. pylori* in the gastric epithelium and in subepithelial extracellular matrix. It seems likely that *H. pylori* may first interact with sialoglycoconjugates and Lewis blood group structures in the lower gastric mucin layer and on epithelial cell surfaces [27]. Binding to cell-surface GAG molecules may trigger cell interactions, as proposed for other GAG-binding pathogens such as trypanosomes and chlamydiae [28, 29].

However, GAG binding to *H. pylori* – together with binding to ECM molecules, including fibronectin, vitronectin, type IV collagen and laminin – may

allow it to become coated efficiently with host molecules capable of preventing attack by professional phagocytes and a strong immune response. This may in part explain why *H. pylori* is a 'slow pathogen' [30, 31], allowing survival for decades in the gastric mucosa of patients with chronic type B gastritis.

In conclusion, *H. pylori* cells interact strongly with HS at a low pH value (4.5) and more than a surface protein are involved in this interaction. According to the biotin-heparin assay, only the protein in the 55–60 kDa region interacts with heparin at a neutral pH (6–7). Although the *H. pylori* cell-surface proteins do not bind bFGF and EGF at pH 5–7, this may occur when the 'bridge' is provided by the heparin molecule.

This work was supported by a grant from the Swedish Medical Research Council (16x-84723), a grant from Magnus Bergvall and the Medical Faculty, Lund, and a grant from the Swedish Institute to M.U. We thank L.-Å. Fransson and Cheng Fang for heparan sulphate (HS-3) and P. Aleljung for useful comments and discussions.

References

1. Lindahl U, Lidholt K, Spillmann D, Kjellen L. More to "heparin" than anticoagulation. *Thromb Res* 1994; **75**: 1–32.
2. Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J, De Clercq E. Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc Natl Acad Sci USA* 1988; **85**: 6132–6136.
3. Butcher BA, Sklar LA, Seamer LC, Glew RH. Heparin enhances the interaction of infective *Leishmania donovani* promastigotes with mouse peritoneal macrophages. A fluorescence flow cytometric analysis. *J Immunol* 1992; **148**: 2879–2886.
4. Choi SH, Stinson MW. Purification of a *Streptococcus mutans* protein that binds to heart tissue and glycosaminoglycans. *Infect Immun* 1989; **57**: 3834–3840.
5. Frevert U, Sinnis P, Cerami C, Shreffler W, Takacs B, Nussenzweig V. Malaria circumsporozoite protein binds to heparan sulphate proteoglycans associated with the surface membrane of hepatocytes. *J Exp Med* 1993; **177**: 1287–1298.
6. Kulane A, Ekre HP, Perlmann P, Rombo L, Wahlgren M, Wahlin B. Effect of different fractions of heparin on *Plasmodium falciparum* merozoite invasion of red blood cells *in vitro*. *Am J Trop Med Hyg* 1992; **46**: 589–594.
7. Lycke E, Johansson M, Svennerholm B, Lindahl U. Binding of herpes simplex virus to cellular heparan sulphate, an initial step in the adsorption process. *J Gen Virol* 1991; **72**: 1131–1137.
8. McClure MO, Moore JP, Blanc DF *et al.* Investigations into the mechanism by which sulfated polysaccharides inhibit HIV infection *in vitro*. *AIDS Res Hum Retroviruses* 1992; **8**: 19–26.
9. Ortega-Barria E, Pereira MEA. Entry of *Trypanozoma cruzi* into eukaryotic cells. *Infect Agents Dis* 1992; **1**: 136–145.
10. Pancake SJ, Holt GD, Mellouk S, Hoffman SL. Malaria sporozoites and circumsporozoite proteins bind specifically to sulfated glycoconjugates. *J Cell Biol* 1992; **117**: 1351–1357.
11. Stephens RS. Molecular mimicry and Chlamydia-host cell interactions. In: Mårdh PA, La Placa M, Ward M (eds) Proceedings of the European Society for Chlamydia Research. Stockholm, Sept, 1992: 6–10.
12. Trybala E, Svennerholm B, Bergström T, Olofsson S, Jeansson S, Goodman JL. Herpes simplex virus type 1 induced hemagglutination: glycoprotein C mediates virus binding to erythrocyte surface heparan sulfate. *J Virol* 1993; **67**: 1278–1285.
13. Vaudaux P, Avramoglou T, Letourneur D, Lew DP, Jozefonvicz J. Inhibition by heparin and derivatized dextrans of *Staphylococcus aureus* adhesion to fibronectin-coated biomaterials. *J Biomater Sci Polym Ed* 1992; **4**: 89–97.

14. Ljungh Å, Wadström T. Binding of extracellular matrix proteins by microbes. *Methods Enzymol* 1995; **253**: 501–514.
15. Wadström T, Ringner M, Valkonen KH. Interactions of microbial lectins and extracellular matrix. *Zentralbl Bakteriol* 1994; **25** Suppl: 73–87.
16. Liang OD, Ascencio F, Fransson L-Å, Wadström T. Binding of heparan sulphate to *Staphylococcus aureus*. *Infect Immun* 1992; **60**: 899–906.
17. Ascencio F, Fransson L-Å, Wadström T. Affinity of the gastric pathogen *Helicobacter pylori* for the N-sulphated glycosaminoglycan heparan sulphate. *J Med Microbiol* 1993; **38**: 240–244.
18. Stephens RS. Molecular mimicry and *Chlamydia trachomatis* infection of eukaryotic cells. *Trends Microbiol* 1994; **2**: 99–101.
19. Hirno S, Utt M, Ringner M, Wadström T. Inhibition of heparan sulphate and other glycosaminoglycans binding to *Helicobacter pylori* by various polysulphated carbohydrates. *FEMS Immunol Med Microbiol* 1995; **10**: 301–306.
20. Soltesz V, Schalen C, Mårdh PA. New selective medium for *Campylobacter pylori*. In: Kaijser B, Falsen E, (eds) Proceedings of the Fourth International Workshop on *Campylobacter* Infection. Gothenburg, Sweden, 1988: 433–436.
21. Fransson L-Å, Havsmark B, Silverberg I. A method for the sequence analysis of dermatan sulphate. *Biochem J* 1990; **269**: 381–388.
22. Doig P, Trust TJ. Identification of surface-exposed outer membrane antigens of *Helicobacter pylori*. *Infect Immun* 1994; **62**: 4526–4533.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* 1970; **227**: 680–685.
24. Fransson L-Å. Structure and functions of cell associated proteoglycans: FIBS 12. Elsevier. 1987: 406–411.
25. Trust TJ, Doig P, Emödy L, Kienle Z, Wadström T, O'Toole P. High-affinity binding of the basement membrane proteins collagen type IV and laminin to the gastric pathogen *Helicobacter pylori*. *Infect Immun* 1991; **59**: 4398–4404.
26. Valkonen KH, Ringner M, Ljungh Å, Wadström T. High-affinity binding of laminin by *Helicobacter pylori*: evidence for a lectin-like interaction. *FEMS Microbiol Immunol* 1993; **7**: 29–37.
27. Borén T, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892–1895.
28. Chen JC-R, Stephens RS. Trachoma and LGV biovars of *Chlamydia trachomatis* share the same glycosaminoglycan-dependent mechanism for infection of eukaryotic cells. *Mol Microbiol* 1994; **11**: 501–507.
29. Ortega-Barria E, Pereira MEA. A novel *T. cruzi* heparin-binding protein promotes fibroblast adhesion and penetration of engineered bacteria and *Trypanozomes* into mammalian cells. *Cell* 1991; **67**: 411–421.
30. Blaser MJ. Pathogenesis of *Helicobacter pylori*-induced inflammation: a “slow” bacterial infection? *Eur J Gastroenterol Hepatol* 1992; **4** Suppl 1: 17–19.
31. Blaser MJ. Hypothesis on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation. *Gastroenterology* 1992; **102**: 720–727.