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Interaction of Lipopolysaccharides of *Helicobacter pylori* with Basement Membrane Protein Laminin

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The ability of hemagglutinating and poorly hemagglutinating strains of the gastroduodenal pathogen *Helicobacter pylori* to bind ^{125}I-radiolabelled laminin was quantitated in a liquid phase assay. Although all strains bound laminin, some hemagglutinating strains were good binders of laminin (maximum of 31% binding), whereas poorly hemagglutinating strains bound intermediate to small amounts of laminin (minimum of 6% binding). Since a hydrophobic component of the bacterium has been reported to be involved in binding of laminin (T. J. Trust, P. Doig, L. Emödy, Z. Kienle, T. Wadström, and P. O'Toole, Infect. Immun. 59:4398–4404, 1991), we investigated the role of lipopolysaccharide (LPS) in the interaction of both types of strains with laminin. Although the extent of inhibition varied among strains, laminin binding to hemagglutinating and poorly hemagglutinating strains was inhibited with homologous and heterologous smooth-form LPS. The ability of heterologous rough-form LPS to produce inhibition comparable to that shown by smooth-form LPS indicated that the O side chain of *H. pylori* LPS was not involved in the interaction. Further inhibition experiments with dephosphorylated LPS, isolated core oligosaccharide, and free lipid A suggested that a phosphorylated structure in the core oligosaccharide mediates the interaction of a hemagglutinating strain of *H. pylori* with laminin, whereas a conserved nonphosphorylated structure in the core oligosaccharide mediates the interaction of a poorly hemagglutinating strain. Furthermore, we showed that the interaction of *H. pylori* LPS with ^{125}I-radiolabelled laminin in a solid phase assay was saturable, specific, and inhibitable with unlabelled laminin. It was postulated that the initial recognition and binding of laminin by *H. pylori* may occur through LPS and that subsequently a more specific interaction with a lectin-like adhesin on the bacterial surface occurs.

Helicobacter pylori is the causal agent of active chronic gastritis in humans, is associated with the development of duodenal ulcers in patients, and may be associated with gastric cancer (27, 33, 34, 42). The bacterium is thus an important human pathogen and has been shown to colonize gastric mucus and to adhere to mucosal cells, especially at intercellular junctions (18, 41). Various binding specificities and putative tissue adhesins of *H. pylori* have been described, including hemagglutination activities with different specificities (9, 21, 49), an *N*-acetylneuraminyllactose-binding fibrillar hemagglutinin (11), binding to GM_3 ganglioside and sulfatides (37, 40), and binding to a species of phosphatidylethanolamine in the gastric mucosa (22), but these interactions alone may not explain the tissue tropism of *H. pylori*.

H. pylori produces cytotoxins and enzymes which may degrade the epithelium, causing cellular destruction (33, 41, 42) and exposure of the underlying basement membrane (16). Ultimately, in such ulcerated tissue the various connective tissue proteins and extracellular basement membrane structures could become exposed and colonized by *H. pylori*. Laminin is a complex, noncollagenous glycoprotein which is important for the structure of the basement membrane by its formation of networks with type IV collagen, entactin/nidogen, and heparan sulfate proteoglycans (53). Laminin bears receptors involved in cell adhesion by normal cells, metastating tumor cells, and certain pathogenic bacteria (15, 23, 43, 44). Surface-exposed proteins of bacteria that bind laminin have been shown to exist on *Streptococcus pyogenes* (45) and uropathogenic *Escherichia coli* (50), and laminin-binding proteins have been identified on *Staphylococcus aureus* (24) and on tissue-invasive *E. coli* (47). Similarly, strains of *H. pylori* have been reported to bind to laminin and also to type IV collagen in a specific and saturable manner (30, 46, 48). The interaction of *H. pylori* with laminin involves a bacterial adhesin recognizing certain sialylated oligosaccharides of the glycoprotein (48). Since the carbohydrate moiety of laminin is believed to play a role in eucaryotic cellular adhesion processes (3, 6), such an interaction of *H. pylori* with laminin could further disorganize the gastric epithelium. Supporting this, *H. pylori* was shown to interfere with the interaction between laminin and a laminin receptor in gastric epithelial membranes (39).

In particular, lipopolysaccharide (LPS) of *H. pylori* can inhibit the interaction between laminin and its receptor on epithelial cells (39), but LPS has received only limited attention as a potential tissue adhesin (30). Chemically, LPSs are composed of a poly- or oligosaccharide moiety and a lipid component, termed lipid A, which anchors LPS in the outer membrane and endows the molecule with its toxic properties (2, 35). High-molecular-weight smooth-form (S-) LPS consists of an O side chain (which is a polymer of repeating oligosaccharide units), a core oligosaccharide, and lipid A, whereas low-molecular-weight rough-form (R-) LPS lacks the O side chain (2). Strains producing LPS lacking the O side chain, because of defects in biosynthesis of LPS, have proven useful in biological and structural studies on the core oligosaccharide and lipid A of those bacteria which normally produce S-LPS (2, 35, 36).

The present study was performed to evaluate the possible

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role of LPS in the binding of *H. pylori* to laminin. Since purified S-LPS was shown to inhibit binding of a number of strains, R-LPS of *H. pylori*, and derivatives therefrom, were employed to identify the molecular basis within the LPS molecule for binding of laminin. The evidence presented suggests that two different mechanisms mediate the interaction of LPS of hemagglutinating and poorly hemagglutinating strains of *H. pylori* with laminin.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *H. pylori* strains for investigation were selected from a collection of hemagglutinating and poorly hemagglutinating strains with differing abilities to bind laminin (48). Four hemagglutinating strains (CCUG 17874, CCUG 19106, 1139, and 12225) and two poorly hemagglutinating strains (CCUG 17875 and 33) were used in these binding studies. *H. pylori* CCUG 17874 and CCUG 17875 (same as NCTC 11637 and NCTC 11638, respectively [National Collection of Type Cultures, London, England]) and CCUG 19106 (strain Pylo 10 [F. Mégraud, Central Regional Hospital, Bordeaux, France]) were obtained from the Culture Collection of the University of Göteborg, Göteborg, Sweden. The other strains were isolates from endoscopic biopsies obtained at the University Hospital, Lund, Sweden. An additional strain, S-24 (provided by D. Danielsson, Medical Center Hospital, Örebro, Sweden), besides CCUG 17874, was used for LPS production. Stock cultures were maintained at −70°C in 15% (vol/vol) glycerol-Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). *H. pylori* strains were grown routinely on blood agar under microaerobic conditions at 37°C for 48 h (29). To obtain *H. pylori* expressing S-LPS, strains were grown in a broth of brain heart infusion (Oxoid Ltd., London, England) containing 2% (vol/vol) fetal calf serum (Oxoid) as described by Moran and Walsh (31).

Isolation of LPS. After pretreatment of bacteria (about 2 g [dry weight]) with pronase (Calbiochem, Los Angeles, Calif.) (4), LPS was extracted from CCUG 17874 and S-24 by the hot phenol-water technique (51). The LPS preparations were purified by treatment with RNase (Sigma Chemical Co., St. Louis, Mo.), DNase II (Sigma), and proteinase K (Sigma) and by ultracentrifugation as described previously (29).

Dephosphorylation of LPS. Preparative dephosphorylation of CCUG 17874 R-LPS was performed by treating LPS (20 mg [dry weight]) with 48% aqueous hydrofluoric acid (Merck, Darmstadt, Germany) at 4°C with constant stirring for 48 h. The resulting suspension was dialyzed against water, and the retentate (dephosphorylated LPS) was freeze-dried.

Preparation of free lipid A and core oligosaccharide. R-LPS of CCUG 17874 was treated with 1% acetic acid at 100°C for 1.5 h (29). Free lipid A was precipitated after centrifugation (3,000 × *g*, 4°C, 30 min), washed with water, freeze-dried, and subsequently solubilized by sonication after the addition of triethylamine to pH 8. The supernatant of the hydrolysate was freeze-dried, redissolved in water, and subjected to gel chromatography on Bio-Gel P-6 (Bio-Rad, Hercules, Calif.; column, 1.2 by 100 cm) to obtain purified core oligosaccharide.

Characterization of LPS and derivatives. The purities of LPS, free lipid A, core oligosaccharide, and dephosphorylated LPS were verified by chemical analyses. Preparations were examined by previously described (29) analytical techniques and procedures for determining phosphate, 3-deoxy-D-*manno*-2-octulosonic acid, neutral sugar, amino sugar, and fatty acids.

Radioiodination of laminin. Laminin, purified from Engelbrett-Holm-Swarm transplantable mouse tumor (AMS Biotechnology, Stockholm, Sweden), was labelled with ^{125}I (sodium salt; specific activity, 14.5 mCi/μg; Amersham International, Amersham, England) according to a modified chloramine-T method using Iodobeads (26). The specific activity of the labelled protein was about 2 × 10^6 cpm/μg. The protein concentration was determined according to the method of Lowry et al. (25).

Binding assays. (i) Liquid phase assays. Binding assays were performed as described previously (48). Briefly, bacterial growth was harvested from cultures, washed once with phosphate-buffered saline (PBS), and finally adjusted to an A_{595} of 1.0 (10^8 cells per ml). The bacterial suspension (100 μl, 10^7 cells) was mixed with 0.55 pmol of ^{125}I-labelled laminin in 0.1 M PBS containing 0.1% (wt/vol) bovine serum albumin (PBS-BSA) and incubated at 20°C for 60 min in air or under microaerobic conditions. Subsequently, bacteria were pelleted by centrifugation (2,000 × *g*, 4°C, 15 min) and washed with PBS. The total radioactivity and the radioactivity of the pellet were determined by conventional scintillation counting in a gamma counter (Wallac, Turku, Finland). Assays were performed in triplicate, unless stated otherwise, and the radioactivity bound in the pellet is expressed as a percentage of the total radioactivity added to the sample.

To assess the role of LPS in laminin binding by *H. pylori* strains, inhibition of bacterial binding to laminin was investigated with purified S- and R-LPS, dephosphorylated R-LPS, core oligosaccharide and lipid A of *H. pylori* CCUG 17874, and R-LPS of *H. pylori* S-24. Inhibition assays were performed by incubation of 0.55 pmol of ^{125}I-laminin in PBS-BSA with different amounts of the inhibitors (5 to 300 μg) at 20°C for 1 h, followed by addition of 100 μl of bacterial suspension (10^7 cells) and incubation for a further 1-h period. The extent of bacterial binding to ^{125}I-laminin was determined as described above. Also, to determine whether LPS-mediated binding to laminin was restricted to *H. pylori*, *E. coli* O26:B6 LPS (Difco Laboratories, Detroit, Mich.) was used as control material in identical inhibition experiments.

The effect of an antiulcer drug on bacterial binding to laminin was investigated with nitecapone [3-(3,4-dihydroxy-5-benzylidiene)-2,4-pentadione], which was kindly provided by P. Pohto, Orion Pharmaceutica, Espoo, Finland. The drug was stored in the dark and before use was suspended in PBS as described by Slomiany et al. (39). Inhibition experiments were performed in three ways. First, bacterial suspension (100 μl, 10^7 cells) was incubated with different concentrations of nitecapone (50 to 500 μg) at 20°C for 2 h in the dark and then incubated with 0.55 pmol of ^{125}I-laminin for 1 h. Second, under the same incubation conditions, 0.55 pmol of ^{125}I-laminin was incubated with different concentrations of nitecapone and subsequently with bacterial suspension. Third, S-LPS of CCUG 17874 (100 μg) was incubated with nitecapone, and the treated LPS was used to inhibit the interaction between ^{125}I-laminin and bacterial suspension. The resulting cell suspensions from these three inhibition experiments were centrifuged (2,000 × *g*, 15 min), the pellets were washed with PBS, and the amount of bound radioactivity was determined.

To determine the roles of both LPS and protein adhesins in laminin binding by *H. pylori*, bacterial suspensions (100 μl, 10^7 cells) were heat treated (80°C, 10 min) or incubated with pronase (5 μg/ml, 37°C, 1 h) and then incubated with 0.55 pmol of ^{125}I-laminin for 1 h. Alternatively, the treated cell suspensions were incubated with nitecapone (200 μg) before incubation with ^{125}I-laminin. The extent of bacterial binding to ^{125}I-laminin was determined as described above.

(ii) Solid phase assays. Microtiter plates (Costar, Cambridge, Mass.) were coated with LPS according to a modification of the method of Freudenberg et al. (17). Briefly, an LPS

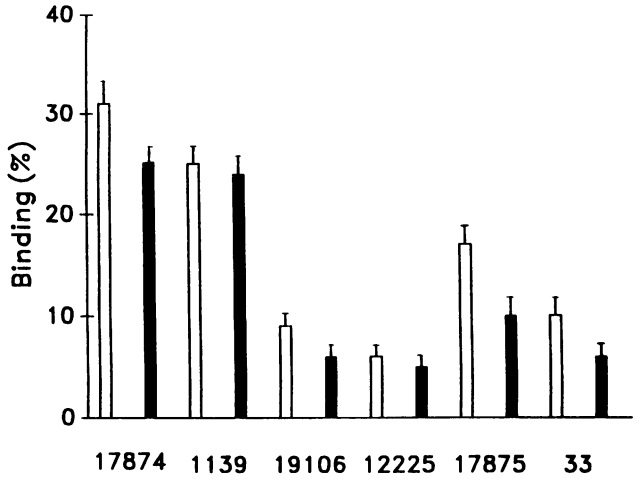

FIG. 1. Effects of S-LPS of *H. pylori* CCUG 17874 on binding of ^{125}I-laminin to *H. pylori* strains. Binding of laminin without preincubation with LPS (■) and after incubation with 100 μg of LPS (□) is shown. Data represent the means of three determinations, and error bars indicate standard deviations.

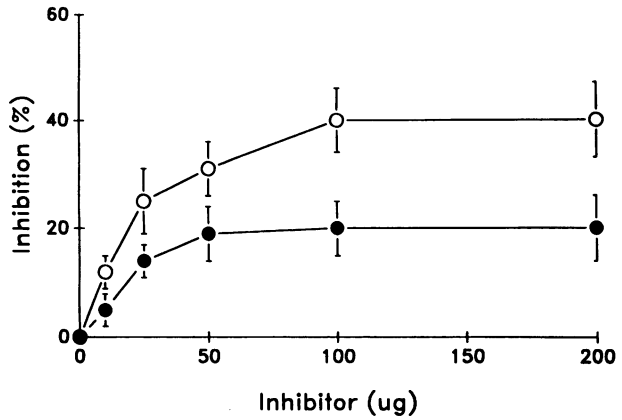

FIG. 2. Effects of concentration of *H. pylori* CCUG 17874 S-LPS on inhibition of binding of ^{125}I-laminin to CCUG 17874 (●) and CCUG 17875 (○) in the liquid phase assay. Data points represent the means of three determinations, and error bars indicate standard deviations.

coating solution (100 μg/ml) was prepared by mixing 100 μl of LPS stock solution (1 mg/ml) with 900 μl of a chloroform-ethanol mixture (1:9, vol/vol). This solution was added to wells and evaporated overnight. Thereafter, wells were washed three times with PBS containing 0.1% Tween 20 (PBS-Tween), further incubated with PBS-BSA at 20°C for 2 h, and finally washed three times with PBS-Tween. Binding of laminin to LPS-coated plates was estimated by incubation of ^{125}I-laminin in coated wells at 20°C for 2 h in air or under microaerobic conditions, followed by three washes with PBS and determination of the amount of bound radioactivity by conventional scintillation counting with a gamma counter.

The specificity of ^{125}I-laminin binding to immobilized LPS was investigated in two ways. First, nonspecific binding of ^{125}I-laminin to wells coated with 1% (wt/vol) BSA was determined and used as a control. Second, inhibition of ^{125}I-laminin binding to LPS-coated wells was determined in the presence of excess unlabelled laminin.

Statistical analysis. Student's *t* test was used to assess the significance of differences between means in binding assays.

RESULTS

Quantitation of laminin binding to *H. pylori* strains. Liquid phase assays were used since they allowed direct assessment of laminin-bacterial interaction and the formulation of inhibition experiments. Identical results were obtained when the assays were performed in air or under microaerobic conditions. Binding of ^{125}I-laminin by the selected hemagglutinating and poorly hemagglutinating *H. pylori* strains varied between 6 and 31% (Fig. 1). Three groups of binding affinities were observed. Some hemagglutinating *H. pylori* strains bound large to intermediate amounts of laminin (CCUG 17874 and 1139); others bound small amounts (CCUG 19106 and 12225). Poorly hemagglutinating strains (CCUG 17875 and 33) that were reported previously to bind large amounts of laminin (48) bound small to intermediate amounts of laminin during the more extensive testing of this study.

Inhibition of laminin binding by *H. pylori* with LPS and LPS derivatives. S-LPS of CCUG 17874, in addition to inhibiting the binding of ^{125}I-laminin to the same strain, inhibited binding of the protein to other hemagglutinating and poorly hemagglutinating strains (Fig. 1). The extent of inhibition varied among strains. Hemagglutinating strains could be divided into two groups: those whose laminin binding was significantly different after inhibition by LPS (CCUG 17874 [$P < 0.01$] and CCUG 19106 [$P < 0.001$]) and those whose inhibition was not statistically significant (1139 [$P > 0.10$] and 12225 [$P > 0.10$]). The greatest inhibition of laminin binding (at most 50%), which was statistically significant ($P < 0.01$), was observed with poorly hemagglutinating strains (CCUG 17875 and 33). Quantitation of the inhibition of laminin binding to *H. pylori* as a function of increasing S-LPS concentration showed that the inhibition was saturable for both hemagglutinating and poorly hemagglutinating strains (Fig. 2), and indicated that the interaction between LPS and laminin involved a limited number of sites.

S-LPS of CCUG 17875 did not inhibit laminin binding to CCUG 17874, although the converse occurred (Fig. 1 and 2). Furthermore, LPS of *E. coli* O26:B26 inhibited binding of laminin to CCUG 17874 in a dose-dependent saturable manner, with maximal inhibition of 20% at 100 μg (Fig. 3) comparable to that obtained with S-LPS of CCUG 17874 (19% at 100 μg [Fig. 2]; not significantly different [$P > 0.10$]). Laminin binding to CCUG 17875, however, was not inhibited by LPS of *E. coli* O26:B26 (Fig. 3).

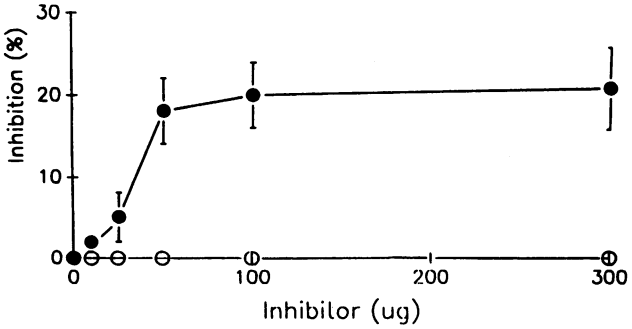

FIG. 3. Inhibition of binding of ^{125}I-laminin to *H. pylori* CCUG 17874 (●) and CCUG 17875 (○) with *E. coli* O26:B26 LPS in the liquid phase assay. Data points represent the means of three determinations, and error bars indicate standard deviations.

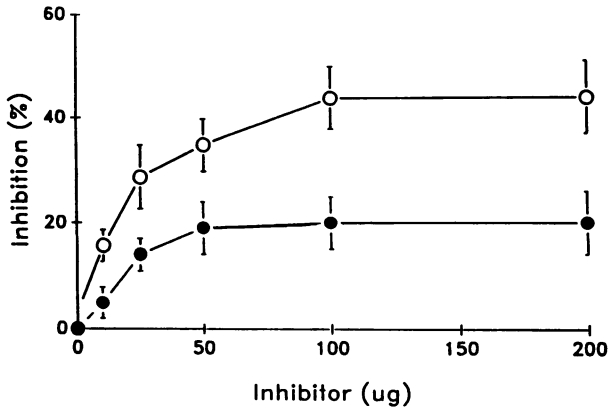

FIG. 4. Effects of concentration of *H. pylori* S-24 R-LPS on inhibition of binding of ^{125}I-laminin to CCUG 17874 (●) and CCUG 17875 (○) in the liquid phase assay. Data points represent the means of three determinations, and error bars indicate standard deviations.

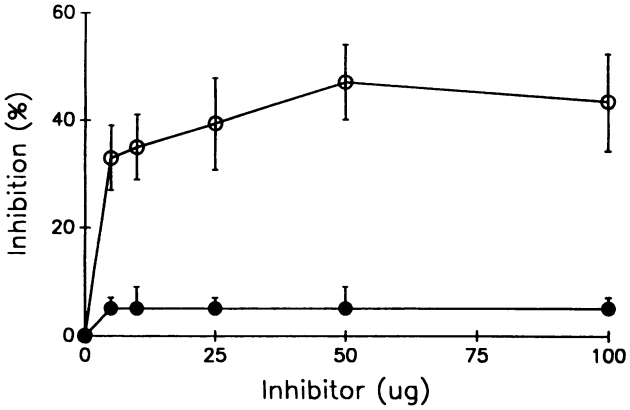

FIG. 5. Inhibition of binding of ^{125}I-laminin to *H. pylori* CCUG 17874 (●) and CCUG 17875 (○) with dephosphorylated R-LPS of CCUG 17874 in the liquid phase assay. Data points represent the means of three determinations, and error bars indicate standard deviations.

The ability of CCUG 17874 S-LPS to cross-inhibit binding of laminin to heterologous strains (Fig. 1 and 2) suggested that structures common to the LPS of a number of *H. pylori* strains could interact with laminin. R-LPS of another *H. pylori* strain, S-24, was used in inhibition experiments to test this hypothesis, since R-LPS lack the O side chain and since the core oligosaccharide and lipid A moieties are the regions of LPS more conserved in structure in strains of a given bacterial species (2, 35, 36). The R-LPS of S-24 inhibited binding of laminin to both hemagglutinating and poorly hemagglutinating *H. pylori* strains (Fig. 4), and the inhibition was comparable to that induced by S-LPS of CCUG 17874. For example, 40% inhibition of binding of CCUG 17875 was observed with 100 μg of S-LPS of CCUG 17874 (Fig. 2), and 43% inhibition of binding was observed with 100 μg of R-LPS of S-24 (Fig. 4) (not significantly different [$P > 0.10$]).

LPS of *H. pylori* strains, including CCUG 17874 and S-24, are phosphorylated (5, 28), and thus the potential role of phosphorylated structures in the LPS-laminin interaction was investigated. Dephosphorylated R-LPS of CCUG 17874 inhibited binding to both hemagglutinating (CCUG 17874) and poorly hemagglutinating (CCUG 17875) strains (Fig. 5) in a saturable manner, similar to data obtained with S-LPS and R-LPS (Fig. 2 and 4). Nevertheless, for CCUG 17874 the maximal inhibition of laminin binding observed with dephosphorylated LPS was 6% (Fig. 5), which was significantly different ($P < 0.001$) from results obtained with native LPS (19% inhibition with 100 μg [Fig. 2]), indicating that phosphorylated structures were involved in the interaction of LPS of this strain with laminin. Although hydrofluoric acid treatment of *H. pylori* LPS can, in addition to dephosphorylation, liberate fucose, it is unlikely that this sugar is involved in the interaction with laminin since fucose is present in small, nonstoichiometric amounts in R-LPS (29), in contrast to that in S-LPS, and no appreciable differences between inhibition of binding by S-LPS and R-LPS were observed (Fig. 2 and 4). For CCUG 17875, however, the maximal inhibition of laminin binding observed with dephosphorylated R-LPS of CCUG 17874 (49% inhibition with 50 μg, [Fig. 5]) was comparable to, and not significantly different from ($P > 0.05$), that obtained with native LPS (40% [Fig. 2]). The latter result showed that some common structure in the R-LPS, but not a phosphorylated structure, was involved in the LPS-laminin interaction with this poorly hemagglutinating strain.

Comparative inhibition assays were also performed with isolated core oligosaccharide and free lipid A to determine the portion of *H. pylori* R-LPS involved in the interaction with laminin. Both core oligosaccharide and free lipid A of CCUG 17874 inhibited binding of laminin to the homologous strain in a dose-dependent, saturable manner (Fig. 6), reflecting the presence of phosphorylated structures in the core oligosaccharide and lipid A (29). In contrast, core oligosaccharide (Fig. 6), but not free lipid A, of the same strain inhibited binding of laminin to CCUG 17875, which showed that a conserved, nonphosphorylated structure in the core oligosaccharide was involved in the LPS-laminin interaction of the latter, poorly hemagglutinating strain.

Effect of nitecapone on LPS-mediated binding of laminin. A series of inhibition experiments which investigated the effects of the antiulcer drug nitecapone on laminin binding by *H. pylori* CCUG 17875 and on the laminin-LPS interaction were

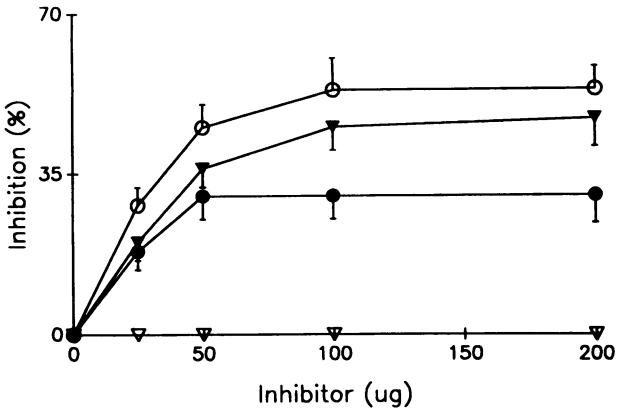

FIG. 6. Inhibition of binding of ^{125}I-laminin to *H. pylori* strains with core oligosaccharide and free lipid A in the liquid phase assay. Shown are the inhibition of binding to CCUG 17874 after preincubation of ^{125}I-laminin with core oligosaccharide (●) and free lipid A (▼) from R-LPS of the same strain and the inhibition of binding to CCUG 17875 after preincubation of ^{125}I-laminin with core oligosaccharide (○) and free lipid A (▽) from R-LPS of CCUG 17874. Data points represent the means of three determinations, and error bars indicate standard deviations.

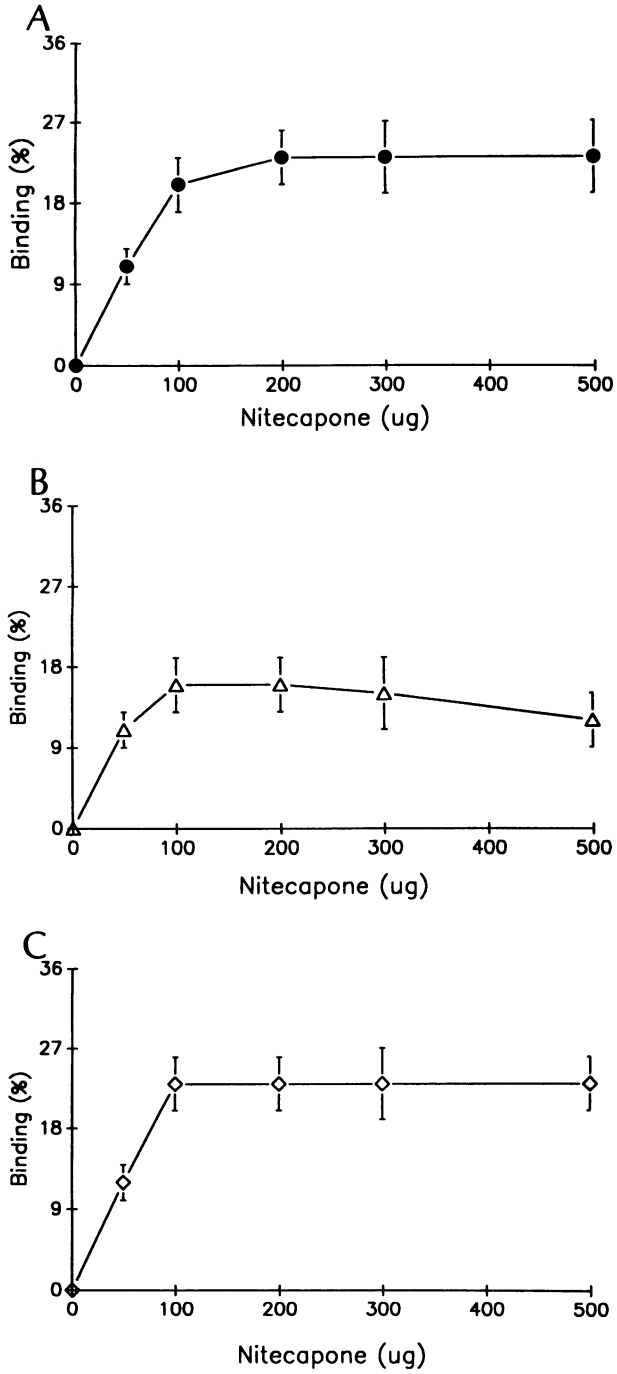

FIG. 7. Effects of nitecapone on binding of ^{125}I-laminin to *H. pylori* CCUG 17875 in the liquid phase assay. (A) ^{125}I-Laminin was pretreated with nitecapone before incubation with bacteria. (B) Bacterial cells were pretreated with nitecapone before incubation with ^{125}I-laminin. (C) S-LPS of CCUG 17874 was pretreated with nitecapone before attempted inhibition of ^{125}I-laminin binding to bacterial cells. Datum points represent the means of three determinations, and error bars indicate standard deviations.

undertaken. Laminin pretreated with nitecapone bound to bacteria in a saturable manner and to the same extent as untreated laminin (Fig. 7A) (not significantly different [$P > 0.10$]). Pretreatment of bacteria with nitecapone, however, reduced laminin binding (e.g., 34% inhibition with 200 μg [Fig. 7B]) (statistically significant [$P < 0.001$]). Furthermore, no reduction of the extent of laminin binding by CCUG 17875 was observed when nitecapone-treated S-LPS of CCUG 17874 was used to inhibit laminin binding to bacteria (Fig. 7C) (not significantly different [$P > 0.10$]). Collectively, these results indicate that nitecapone interferes with the binding of laminin mediated by LPS.

Further experiments using nitecapone were undertaken to investigate the role of protein adhesins, in addition to LPS, in laminin binding by *H. pylori*. Binding of laminin to CCUG 17874 and CCUG 17875 was reduced by 85 and 65%, respectively, when strains were heat treated (80°C, 10 min). Treatment of bacterial cells with pronase reduced laminin binding by 80 and 60%, respectively. Both results suggest the involvement of protein adhesins in binding of laminin. In addition, when heat-treated or enzyme-treated bacteria were incubated with nitecapone (200 μg), complete inhibition of laminin binding was observed, indicating the involvement of both LPS and protein adhesins in binding of laminin.

Solid phase analysis of laminin binding. The molecular interaction of LPS with laminin was investigated further to confirm the specificity of the interaction and to determine its binding constant. Since it was not possible to formulate an assay system with immobilized laminin to give sensitive detection of bound LPS, an assay using LPS-coated wells of microtiter plates with detection of bound ^{125}I-laminin was utilized. Binding of ^{125}I-laminin to immobilized S-LPS of CCUG 17874 showed saturation (Fig. 8A). Scatchard plot analysis (38) gave a straight line indicative of a single class of binding interaction with a binding constant of 2.5 nM (Fig. 8B). The specificity of the interaction was further demonstrated by inhibition of ^{125}I-laminin binding to LPS by unlabelled laminin in a concentration-dependent manner (Fig. 9). Identical results were obtained when assays were performed in air or under microaerobic conditions.

DISCUSSION

The precise role that attachment plays in the pathogenesis of *H. pylori* has not, to date, been unequivocally established, but the bacterium shows a strong ability to adhere in various in vitro test systems (10, 13, 14, 32) and can adhere to gastric mucosal cells in vivo (18). Furthermore, the adhesive process is apparently complex, since the organism produces a variety of different adhesins (9, 11, 21, 22, 30, 37, 40, 49). It has been suggested that this multiplicity of adhesins may reflect that *H. pylori* adherence is a multistep process involving different adhesion and recognition interactions at different stages of the process (7) and that *H. pylori* expresses several adhesins mediating adherence to various targets in gastric tissue (42). The latter possibility is supported by the finding that in vivo not all *H. pylori* cells are in direct contact with the gastric mucosal epithelium, but rather some are found within the mucus gel overlying the epithelium, whereas others can be observed in close proximity to intercellular junctions (18, 42).

The capacity of *H. pylori* strains to bind to basement membrane laminin is unlikely to be involved in the initial colonization of gastric mucosal cells (46), since specific primary adhesins recognize receptors in the mucus layer and on the epithelial cell surface (11, 22, 37, 40, 41). Nevertheless, this binding property would assume major importance once the basement membrane became exposed. This exposure may result from the microbial activity of *H. pylori* (33, 39, 41, 42) or feeding (52). Electron microscopy studies have shown that *H. pylori* can disrupt the epithelial cells after colonization of the

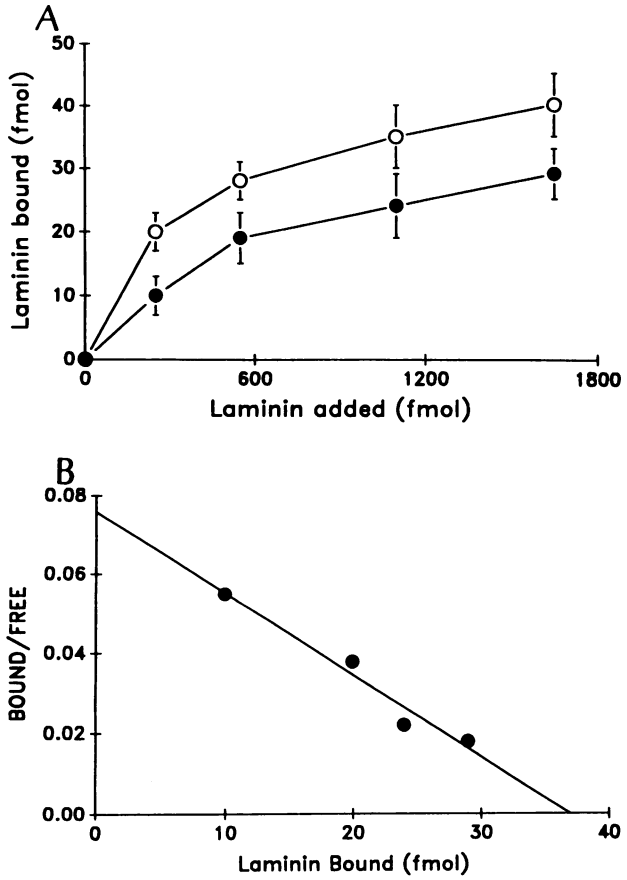

FIG. 8. Binding of ^{125}I-laminin to immobilized S-LPS of *H. pylori* CCUG 17874 in the solid phase assay. (A) The total amount of ^{125}I-laminin bound to immobilized LPS (○) was determined, and subsequently, after subtraction of nonspecific binding of ^{125}I-laminin to BSA-coated surfaces, the amount of laminin bound specifically to LPS (●) was estimated. Data points represent the means of three determinations, and error bars indicate standard deviations. (B) Scatchard plot of specific binding data.

gastric mucosa, leaving the underlying basement membrane bare (16). Alternatively, a high rate of cell turnover in the gastric epithelium may expose laminin and other extracellular matrix components for the bacterium to interact with (29, 46). Tissue damage may be further enhanced by backflow of gastric secretions into these microlesions, and thus laminin binding may enable the pathogen to survive in chronically inflamed tissue.

In addition to binding to gastric mucosal cells in vivo, different *H. pylori* strains exhibit different hemagglutination specificities in vitro (49), further emphasizing a multiplicity of adhesin production. However, the relevance of hemagglutinins to pathogenesis of *H. pylori* in vivo remains unclear, and the adherence mechanisms of poorly hemagglutinating strains were, to date, unknown. In contrast to the diversity of hemagglutination specificities, conserved binding of laminin and collagen type IV by *H. pylori* strains has been reported (46), but the conserved nature of this binding between strains has been disputed (30). Upon examination of laminin binding to various *H. pylori* strains in the present study, we found that hemagglutinating strains could be divided into high- and low-level binders of laminin, whereas poorly hemagglutinating strains were low- to intermediate-level binders of laminin, suggesting a multiplicity of bacterial-glycoprotein interactions.

A hydrophobic surface component of *H. pylori* is apparently involved in the interaction with laminin (46). Initially, binding of *H. pylori* to laminin was attributed to an afimbrial 19.6-kDa protein adhesin (7), but further characterization of the protein revealed that it was a cytosolic iron-binding protein whose adherence to laminin was due to nonspecific hydrophobic interactions (8). Nevertheless, since LPS is relatively hydrophobic and surface exposed, it is a potential candidate for involvement in the interaction. The observed ability of *H. pylori* LPS to inhibit binding of hemagglutinating and poorly hemagglutinating strains to laminin supports this hypothesis, although the extent of inhibition of binding with LPS also supports the involvement of another molecular interaction in the adhesion process. In addition, denaturation experiments indicated the involvement of both protein adhesins and LPS in the interaction with laminin. Although previously we observed that an LPS preparation from NCTC 11637 (same as CCUG 17874) completely inhibited laminin binding by the same strain (30), further analysis has revealed that the LPS preparation was contaminated by protein and hence potentially by the other adhesin involved in laminin binding.

On the basis of the results obtained in the earlier parts of this study, two strains, one hemagglutinating and a high-level binder of laminin (CCUG 17874) and the other poorly hemagglutinating and an intermediate-level laminin binder (CCUG 17875), were selected for more detailed studies of LPS-laminin interactions. It is interesting that inhibition by CCUG 17874 LPS, even though the LPS was from a heterologous strain, was greater in the poorly hemagglutinating strain than in the hemagglutinating one, emphasizing differences between the strains in bacterial-laminin interaction. Supporting this conclusion, LPS of CCUG 17875 did not inhibit binding of laminin to the heterologous hemagglutinating strain. The ability of *E. coli* LPS to inhibit binding of the hemagglutinating strain, but not the poorly hemagglutinating strain, confirmed that different modes of binding of laminin by LPS existed in the two *H. pylori* strains.

Nevertheless, even though two separate modes of interaction of LPS with laminin were apparent in the hemagglutinating and poorly hemagglutinating strains, it was possible to cross-inhibit binding of both types of strains with heterologous

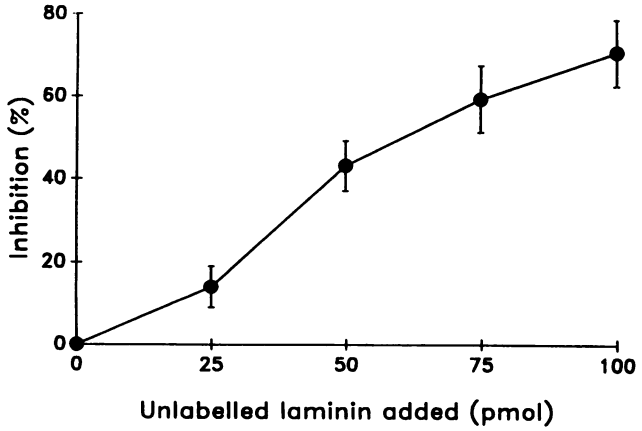

FIG. 9. Inhibition of binding of ^{125}I-laminin to immobilized S-LPS of *H. pylori* CCUG 17874 with unlabelled laminin in the solid phase assay. Data points represent the means of three determinations, and error bars indicate standard deviations.

S- and R-LPS. Thus, a relatively conserved region (core oligosaccharide or lipid A), but not a strain-specific region (O side chain), in LPS is involved in the interaction.

Comparative inhibition experiments with dephosphorylated R-LPS showed that phosphorylated structures in the core of *H. pylori* LPS were involved in the interaction of the hemagglutinating strain, but not the poorly hemagglutinating strain, with laminin. Further, the presence of phosphate, phosphorylethanolamine, and pyrophosphorylethanolamine as substituents in the core oligosaccharide and lipid A of LPS of members of the family *Enterobacteriaceae* (2, 35, 36) explains the ability of *E. coli* LPS to cross-inhibit the interaction of the hemagglutinating strain with laminin. Although both isolated core oligosaccharide and free lipid A inhibited binding of laminin by the hemagglutinating strain, it is unlikely that phosphorylated structures in lipid A mediate the interaction between bacterial cells and laminin, since lipid A is embedded in the outer membrane of gram-negative bacteria and is covered by the sugars of the inner core oligosaccharide (20). Only core oligosaccharide inhibited binding of the poorly hemagglutinating strain. These results, therefore, suggest that a phosphorylated structure in the core oligosaccharide mediates the interaction of LPS of the hemagglutinating strain with laminin, whereas in contrast, a conserved nonphosphorylated structure in the core oligosaccharide mediates the interaction of the poorly hemagglutinating strain.

Of particular interest is that although the LPS-laminin interactions of the hemagglutinating and the poorly hemagglutinating strains were mediated by different mechanisms, LPS of the hemagglutinating strain was able to cross-inhibit the interaction of the poorly hemagglutinating strain with laminin. It would therefore appear that despite the LPS of the hemagglutinating strain possessing the responsible structure in its core oligosaccharide, it is masked on the surface of that bacterial strain and cannot mediate the interaction of the hemagglutinating strain with laminin.

In addition to a hydrophobic component (46), a lectin-like bacterial adhesin (48) has been implicated in the binding of laminin by *H. pylori*. The incomplete inhibition of laminin binding to *H. pylori* by LPS is therefore in agreement with the existence of a second type of interaction between laminin and the bacterial surface in the adhesion process. Thus, initial recognition and binding of *H. pylori* to laminin may occur through LPS, and subsequently a more specific interaction with a lectin-like protein adhesin on the bacterial surface may occur. Likewise, McSweegan and Walker (28) showed that both LPS and another surface structure, possibly flagella, mediated adhesion of *Campylobacter jejuni* to epithelial cells. A similar adhesion mechanism involving LPS and protein receptors on epithelial cells has been described for *Shigella flexneri* (19), and LPS of *Actinobacillus pleuropneumoniae* has been identified as the molecule mediating adhesion of the pathogen to porcine trachea (1). However, the interaction of *H. pylori* LPS with laminin is the first characterization of such an interaction by a spiral, gram-negative, mucus-colonizing bacterium.

Slomiany et al. (39) demonstrated that *H. pylori* cells, and in particular *H. pylori* LPS, inhibit the interaction between a laminin receptor in gastric epithelial cells and laminin. The subsequent disruption of epithelial cell-basement membrane interaction by LPS would explain the development and continuance of lost mucosal integrity during *H. pylori* infection (30). The antiulcer drug nitecapone, however, abolishes the inhibitory effect of LPS on the epithelial cell-laminin interaction (39). Our observation that nitecapone-treated LPS no longer inhibited binding of laminin to *H. pylori* indicates that the drug also interferes with the adhesion mediated by LPS. Furthermore, the incomplete inhibition of bacterial adherence by the drug is consistent with a second molecular interaction in the adhesion process.

Binding of laminin to *H. pylori* cells has been shown to be saturable and of high affinity (30, 46, 48). Likewise, we demonstrated that the interaction of laminin with LPS in the solid phase assay was saturable, specific, and inhibitable with unlabelled protein. The binding constant of 2.5 nM for the LPS-laminin interaction lies within the range of 8.5 pM to 7.9 nM that has been calculated for the interaction of laminin with *H. pylori* (46, 48). Also, the LPS-laminin binding constant is slightly higher than the 2.9 nM laminin binding constant reported for *S. aureus* (24) but significantly higher than the values of 40 to 80 nM reported for streptococci (44, 45). However, the protein adhesin with lectin-like properties involved in the interaction between laminin and *H. pylori* remains to be identified. Of interest is that the *H. pylori* lectin-like adhesin recognizes *N*-acetylneuraminyllactose oligosaccharides of laminin (48), and binding to cell surfaces with such oligosaccharides has been demonstrated in vitro (10). Furthermore, an *N*-acetylneuraminyllactose-binding hemagglutinin of *H. pylori* has been described and cloned by Evans et al. (11, 12); this hemagglutinin thus represents a potential candidate for the other adhesin involved in laminin binding.

In summary, the interaction of *H. pylori* LPS with laminin is, to our knowledge, the only binding of laminin by a nonproteinaceous bacterial surface component identified to date. Furthermore, the interaction of LPS with extracellular matrix proteins may represent a previously unrecognized group of surface interactions produced by significant gastrointestinal pathogens of humans. The in vitro laminin adherence assay should prove useful in further characterizing *H. pylori* adhesin-receptor interactions and for identifying potentially therapeutic drugs against *H. pylori* that inhibit adherence of this human pathogen.

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