A Human Domain Antibody and Lewis$^b$ Glycoconjugate That Inhibit Binding of Helicobacter pylori to Lewis$^b$ Receptor and Adhesion to Human Gastric Epithelium

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Increasing antibiotic resistance has prompted development of alternative approaches to antimicrobial therapy, including blocking microbial adhesion to host receptors. The BabA adhesin of Helicobacter pylori binds to fucosylated blood group antigens, such as the Lewis$^b$ antigens in human primate gastric mucosa. We have isolated a human domain antibody specific for BabA that inhibits binding of BabA to Lewis$^b$ and prevents adhesion of H. pylori to human gastric epithelium. In addition, Lewis$^b$ oligosaccharides covalently linked to poly-D-lysine inhibited BabA binding to Le$^b$. The poly-D-lysine-Le$^b$ hexasaccharide and an Le$^b$ human serum albumin conjugate not only inhibited adherence of H. pylori to gastric epithelium but also displaced adherent bacteria when added to human stomach sections. Combinations of Le$^b$ and sialyl Le$^b$ or domain antibody 25 and sialyl Le$^b$ acted synergistically. Domain antibody 25 inhibitor may have potential for prophylactic use and, in combination with Le$^b$ glycoconjugates, therapeutic use in treatment of drug-resistant H. pylori infection.

Helicobacter pylori colonizes the gastric mucosa and causes serious gastroduodenal disease [1]. The grade of gastritis is in part dependent on factors involved in bacterial attachment including the H. pylori blood group antigen-binding adhesin BabA [2]. Expression of BabA correlates with overt disease, such as peptic ulceration and adenocarcinoma [3, 4]. In a transgenic mouse, expression of the human Lewis$^b$ antigen (Le$^b$) facilitated adherence of H. pylori to the gastric epithelium and increased host inflammatory responses, compared with that of the wild-type mouse [5]. An additional H. pylori adhesin, the sialic acid–binding adhesin (SabA), was identified with specificity for sialyl-Lewis$^x$ antigen (sLex) [6]. Expression of sLex in gastric mucosa is much increased after induction of chronic inflammation. SabA binds to sLex and other similar α2,3-sialylated glycoconjugates and provides an additional adherence mechanism during life-long and persistent H. pylori infection.

Treatment of infection involves a combination of antibiotics and a proton-pump inhibitor (triple or quadruple therapy), but the prevalence of antibiotic resistance is increasing [7, 8]. Development of compounds that inhibit interaction of H. pylori with host receptors may provide an alternative approach to prevent infection. Neoglycoproteins of the Le$^b$ or sLe$^b$ oligosaccharides conjugated to human serum albumin (HSA) inhibited BabA- or SabA-mediated adhesion, respectively,
of *H. pylori* to gastric mucosa in vitro [6]; however, the costs of production may preclude their development as anti-infective agents. Naturally occurring glycoconjugates in human and porcine milk also prevent *H. pylori* adhesion in vitro [9, 10], and studies using porcine milk showed inhibition in vitro and in a murine model of infection [11].

In the present study, we investigated novel inhibitors of *H. pylori* adhesion, domain antibodies directed against BabA, and 2 glycoconjugates of Le^\text{e}\textsuperscript{b} multivalently coupled to poly-d-lysine. Domain antibodies correspond to the variable regions of either the heavy (V_\text{H}) or light (V_\text{L}) chains of human antibodies [12–14]. We describe testing of V_\text{K} domain antibodies with specificity for BabA in a variety of systems for inhibition of *H. pylori* adhesion. In contrast to conventional antibodies, domain antibodies are well expressed in bacterial, yeast, and mammalian cell systems and may be well suited for use as compounds to prevent *H. pylori* attachment to gastric epithelium.

**MATERIALS AND METHODS**

Purified BabA protein from *H. pylori* 17875 was kindly provided by Thomas Borén (University of Umeå). HSA glycoconjugates of Le^\text{e}\textsuperscript{b} and Lewis^\text{a}\textsuperscript{b} (Le^\text{e}) were from IsoSep AB (Tullinge, Sweden) and contained ~20 mol Le^\text{e}\textsuperscript{b} or Le^\text{e} per mol of HSA, respectively.

**H. pylori Isolates**

*H. pylori* wild-type strain J99, single babA and sabA mutant strains, and the double babAsabA mutant strain were kindly provided by Thomas Borén [6]. *H. pylori* strains J99 (and mutants), NCTC 11637, and CCUG17875 were grown for 2–3 days on Columbia agar plates supplemented with 5% horse blood (Oxoid) at 37°C under micro-aerobic conditions. Bacterial cell numbers in suspension were determined by optical absorbance at 600 nm (Ultrorspec II; LKB) and were correlated with total viable counts.

**Fluorescent Labelling of *H. pylori* Isolates**

*H. pylori* isolates (1 × 10^7 cells/L) were labeled with fluorescein isothiocyanate isomer I (Sigma) and were resuspended in phosphate-buffered saline (PBS). Labeling was confirmed by flow cytometry (FACSCalibur; Becton Dickinson).

**Le^\text{e}\textsuperscript{b} Glycoconjugates**

Lactose β1-O-(3-benzyloxy carbonyl amino) propane was selectively protected in the 3'-position with p-methoxybenzyl chloride–dibutyltin oxide. The remaining hydroxyls were benzylation with benzyl bromide–sodium hydroxide under phase transfer conditions. The p-methoxybenzyl group was removed using ceric ammonium nitrate under standard conditions. The resulting disaccharide acceptor was glycosylated with 1-thioethyl 3,4,6 tri-O-acetyl N-trichlorooxycarbonyl glucosamine with use of N-iodosuccinimide–trifluoromethanesulfonic acid activation. The trichloro oxycarbonyl group was replaced with an acetyl group by using zinc dust and then acetic anhydride. The O-acetyl groups were removed with sodium methoxide. The modified trisaccharide was then converted to Le^\text{e}\textsuperscript{b} hexasaccharide derivative [15]. The resulting Le^\text{e}\textsuperscript{b} hexasaccharide with a free amine group at the reducing end of a 3 carbon chain was conjugated to poly-d-lysine: 3 mg was reacted with a large molar excess (35 mg) of pimelic acid dipentafluorophenyl ester, and the resulting extended chain ester activated hexasaccharide was purified by reverse-phase chromatography, rapidly isolated by low-temperature high-vacuum evaporation, dissolved in dimethylsulfoxide, and reacted with a solution of poly-d-lysine (2 mg) in dimethylsulfoxide (at room temperature for 2 days). The final product was diluted with water and was freeze-dried, yielding high-purity multivalent Le^\text{e}\textsuperscript{b} hexasaccharide conjugated to poly-d-lysine at an estimated loading of 0.5 mg oligosaccharide per mg of poly-d-lysine.

Le^\text{e}\textsuperscript{b} tetrasaccharide poly-d-lysine conjugate was prepared from N-acetyl glucosamine β1-O-(3-benzyloxy carbonyl amino) propane with use of chemical transformations similar to those applied to the hexasaccharide analogue. Four milligrams of multivalent Le^\text{e}\textsuperscript{b} tetrasaccharide conjugated to poly-d-lysine at an estimated loading of 0.7 mg oligosaccharide per mg of poly-d-lysine was prepared. 3'-Sialyl Le^\text{e}\textsuperscript{b} tetrasaccharide (sLe^\text{b}) was synthesized as described elsewhere [16].

**Production and Screening of Domain Antibody Library**

The domain antibody phage libraries were based on a single human antibody framework for V_\text{H} (DP47/JH4b) and for V_\text{L} (DPK4/Jk1). Amino acid side chain diversity was introduced through DNA nucleotide diversification at positions naturally diversified in the mature human repertoire. Selections against passively coated BabA from *H. pylori* strain CCUG17875 were performed essentially as described elsewhere [17], with the exception that bound phage were eluted at room temperature for 10 min with 500 mL of PBS containing 1 mg/mL trypsin (Sigma-Aldrich) supplemented with 0.1 mmol/L calcium chloride (Sigma-Aldrich). After selection rounds 2 and 3, the enriched domain antibody genes were subcloned into the soluble domain antibody expression vector pDOM5 and used to transform *Escherichia coli* HB2151 for further analysis.

Supernatents (100 μL) from individual *E. coli* HB2151 clones transformed with domain antibody DNA were analyzed for BabA binding activity with use of enzyme-linked immunosorbent assay using 9E10 antibody (anti-myc; Sigma-Aldrich) to detect bound domain antibody. Forty-three unique binding clones (by DNA sequence) were grown at 37°C with shaking at 250 rpm in 2× TY (50 mL) supplemented with 50 mg/mL carbenicillin and 0.1% glucose. Isopropyl-β-D-thiogalactopyranoside (1 mmol/L) was added when the optical density at 600 nm reached 0.8, and cultures were incubated overnight at 30°C with shaking. Cells were pelleted by centrifugation (3450 g for

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10 min), and supernatants (50 mL) were mixed by rotation at 50 rpm with 200 μL of protein-A-sepharose (Sigma-Aldrich) for V, domain antibodies or protein-A streamline (GE Healthcare) for V, domain antibodies at room temperature for 1 h. The resins were recovered by centrifugation (220 g for 1 min), transferred to a 96-well paper-filter plate (Whatman), and then washed twice with 0.5 mol/L sodium chloride in PBS (2 × 1 mL) and PBS (2 × 1 mL). Domain antibodies were eluted with 0.1 mol/L glycine (pH, 2.0; 210 μL) and were neutralized with 1 mol/L Tris–hydrochloric acid (pH, 8.0; 40 μL).

Bacterial Binding Assays

Binding of whole cells and BabA to Leb-HSA was determined by surface plasmon resonance (SPR) using the Biacore X (whole cells and BabA protein) or Biacore J (whole cells only; GE Healthcare). Leb-HSA was immobilized on a CM-5 chip with use of amine coupling (BIA Applications Handbook; GE Healthcare). Similar amounts of either HSA or Leb-HSA were immobilized to the reference flow cells.

**Binding of intact H. pylori.** Intact cells (1 × 10⁶ colony-forming units [cfu] per mL) of H. pylori strain J99, single babA and sabA mutant strains, or the double babAsabA mutant strain [6] were injected for 5 min at a flow rate of 5 μL/min over immobilized Leb-HSA in HEPES-buffered saline. The sensorchip was regenerated by injection (1 min) of 100 mmol/L diethy lamine.

Binding of BabA to Leb-HSA was measured by injection of BabA (25–200 nmol/L) in HEPES-buffered saline containing 0.005% (weight/volume) Tween 20 supplemented with 10 mmol/L N-octylglucoside to maintain BabA in solution. For kinetic analyses, BabA was injected at different flow rates (5–60 μL/min) and with contact times of 10 s to 3 min. The sensorchip surface was regenerated by a pulse (1 min) of 2 mol/L potassium thiocyanate.

**Screening of inhibitors.** The effect of domain antibodies on BabA binding to Leb-HSA was determined by coinjection of BabA (5 nmol/L) and domain antibodies (1 μmol/L) with protein L (1 μg/mL; to form multimeric domain antibodies). Domain antibody HEL4 (with specificity for hen egg lysozyme) was used as a negative control. Similarly, Leb neoglycoconjugates were coinjected with BabA (25–50 nmol/L) at 5 μL/min for 4 min.

The effect of domain antibody 25 on binding of H. pylori to Leb-HSA was determined by coinjection of domain antibody 25 (1 μmol/L) with protein L (1 μg/mL) in the presence of 5 × 10⁴ cfu/mL of H. pylori strains CCUG17875, J99, and 11637. Domain antibody 9 was used as a noninhibitory control.

Histological Sections of Human Stomach

Formalin-fixed Leb-expressing H. pylori–negative biopsy samples of human stomach were cut into 5-μm sections with use of a Leica SM2400 rocking microtome and were collected on Vectabond-coated slides (Vector Laboratories). Antigen expression was optimized by boiling (15 mL blocking buffer PBS, 2% bovine serum albumin, and 0.05% Tween-20 [PBST] per slide before washing (3 times). Suspensions of fluorescein isothiocyanate isomer I H. pylori (1 × 10⁴ cfu/mL) and inhibitor with protein L (1 μg/mL; for domain antibodies only) were incubated with gentle shaking for 2 h and washed with blocking buffer. Propidium iodide (200 μL of 5 μg/mL) was added to each slide for 3 min. Slides were washed twice in PBST, air-dried, and mounted with Vectashield (V-1000; Vector Laboratories). All incubation and washing steps were performed in the dark at room temperature. Combinations of inhibitors were also assayed.

**Removal of bound H. pylori.** To measure removal of H. pylori, inhibitors were incubated with tissue samples to which H. pylori was attached. Sections were then washed 3 times in PBST, stained with propidium iodide, washed in PBST, and mounted as previously described.

Quantification of Binding

Sections were observed using a Laser Scanning Confocal Microscope (Zeiss: Axiovert 100 TV) with a ×20 Zeiss LD-ACHR objective. Digital images of sections were analyzed and quantified using Metamorph software [18]

RESULTS

**Identification of domain antibodies with specificity for BabA.** Enzyme-linked immunosorbent assay screening of the phage display domain antibody libraries identified 43 unique (by DNA sequence) domain antibodies with binding activity for BabA.

**Binding of H. pylori and BabA in SPR assays.** Binding of wild-type H. pylori strain J99, single babA and sabA mutants, and the double babAsabA mutant to Leb-HSA were measured. Compared with the wild-type J99, both the single babA and the double babAsabA mutants bound at very low (<10%) levels. The sabA mutant bound at ∼70% of the level of J99 (Figure 1A), which may reflect an effect of SabA on surface expression of BabA. Purified BabA protein bound to Leb-HSA, showing dose dependence (Figure 1B), and was inhibited by Leb-HSA but not by Leb-HSA (Figure 1C). In subsequent experiments, Leb-HSA was coupled to the reference flow-cell. BabA binding to HSA-Leb could not be fitted to the Langmuir binding model using Bia-evaluation software but fit better to a linked-reaction
Figure 1. Surface plasmon resonance analyses of BabA binding to Lewis\textsuperscript{a} antigen (Le\textsuperscript{a})–human serum albumin (HSA). Le\textsuperscript{a}-HSA was immobilized on the sensorchip surface, and binding was determined of intact \textit{Helicobacter pylori} cells or of BabA (with or without inhibitors) in fluid phase. Panels show superimposed sensorgrams. a, Binding of wild-type \textit{H. pylori} (J99) and of single mutants lacking SabA or BabA and the double mutant lacking both. Sensorgrams of duplicate injections of both are shown. Arrows indicate start and finish of injections. b, Fluid-phase BabA was injected at 4 different concentrations. Arrow indicates the end of injection (start at time 0). Sensorgrams were fitted to a 2-step linked reaction, and the fit is indicated by a broken line. c, Fluid-phase BabA injected with Le\textsuperscript{a}-HSA or Le\textsuperscript{y}-HSA. d, Fluid-phase BabA was injected at 60 $\mu$L/minute over immobilized Le\textsuperscript{a}-HSA. Fitting of data to sensorgrams using the Biaevaluation program is consistent with a 2-step conformational-change model. Fitted data are indicated by a broken line. e, Effect of altering contact time on dissociation of Le\textsuperscript{a}-HSA complex. Superimposed sensorgrams of different injection times show differences in dissociation rate. f, Dissociation regions of sensorgrams from panel e show decreased dissociation with increased contact time. RU, resonance units.
model (Figure 1B). For improved kinetic analyses, BabA was injected at a higher flow rate (60 μL/min) over the sensorchip surface with relatively low levels of immobilized Leβ-HSA. Representative sensorgrams indicated an improved fit to the linked-reaction model (Figure 1D). To investigate this further, the contact time of BabA was varied (10 s, 30 s, and 60 s), and under these conditions, the apparent dissociation rate decreased with increasing contact time (Figure 1E and 1F), which is consistent with the linked-reaction fitting model. In the absence of evidence for bivalent binding of BabA, a 2-step conformational-change model was used to describe formation of the BabA-Leβ-HSA complex. The apparent $K_a$ of $\sim 2 \times 10^{-7}$ mol/L (calculated from sensorgrams generated under conditions used for those shown in Figure 1D) derives from an initial binding step ($k_{on}$, $2.5 \times 10^4$ mol/L$^{-1}$s$^{-1}$), followed by a slower conformational change ($k_{off}$, $\sim 2 \times 10^{-2}$ s$^{-1}$), to form a relatively stable complex ($k_{d2,s}$).

Inhibitory activity of domain antibodies for BabA binding to Leβ. BabA (5 nmol/L) was coinjected with domain antibodies (1 μmol/L) in the presence of protein L to measure inhibition. Three domain antibodies (25, 26, and 28) showed complete inhibition of BabA binding to Leβ (Figure 2A). Monomeric domain antibodies (no protein L) did not inhibit. In some instances, the signal was increased when BabA was injected in the presence of domain antibody (eg, domain antibodies 16, 27, 34, and 35), which may have been attributable to binding of complexes of BabA and noninhibitory domain antibody to immobilized Leβ-HSA. The most potent inhibitor, domain antibody 25, completely inhibited binding at 50-fold molar excess over BabA (Figure 2B). In the whole cell binding assay with H. pylori CCUG17875 (Figure 2C), domain antibody 25 reduced binding by $\sim 50$%; domain antibody 26 was less effective ($\sim 25$% inhibition), and a small increase in signal was observed in the presence of domain antibody 9. Domain antibody 25 also inhibited binding of H. pylori NCTC 11637 and, most effectively, J99 (Figure 2C).

Inhibitory activity of poly-D-lysine conjugates of Leβ in SPR assays. Poly-D-lysine conjugates of Leβ tetrasaccharide (Leβ[tet]PL) and hexasaccharide (Leβ[hex]PL) were investigated as inhibitors of BabA binding to Leβ-HSA. Leβ(hex)PL almost completely inhibited binding at molar equivalence to BabA (Figure 2D) and compared well with Leβ-HSA (Figure 1C). Leβ(tet)PL was less effective, requiring 4-fold molar excess of inhibitor to reduce binding similarly (Figure 2E).

Inhibitory activity of domain antibodies in stomach tissue model. Domain antibodies 25 and 28 (with protein L) were assessed for their ability to inhibit adhesion of H. pylori 11637 to histological sections of Leβ human stomach and were compared with the negative control domain antibody HEL4. Domain antibody 28 inhibition was no greater than that of domain antibody HEL4. In contrast, domain antibody 25 inhibited dose-dependently, with 73% inhibition at 100 μg/mL ($\sim 8$ μmol/L) (Figures 3A and 4A and 4B). This was significantly higher than that achieved with the control ($P < .01$) and, compared with binding in the absence of domain antibodies, was very highly significant ($P < .001$). Domain antibody HEL4 inhibited adhesion of H. pylori at 10 μg/mL. The inhibition of binding of H. pylori by the irrelevant domain antibody at low concentrations may be attributable to some unexpected conformational homology between BabA and hen egg lysozyme or to binding of the domain antibody to Leβ. The latter is more likely because lysozyme is a basic protein and binds to polyanions. The reason for the apparent reduction in inhibition with increasing concentrations by domain antibody HEL4 remains unexplained.

Inhibitory activity of Leβ glycoconjugates in stomach tissue model. Leβ glycoconjugates and free sLeβ were tested for inhibition of H. pylori adhesion to stomach. Leβ(hex)PL was the most potent inhibitor, showing a dose response with maximum inhibition of 92% at 250 μg/mL ($P < .01$) (Figure 3B). Leβ-HSA also inhibited in a dose-dependent manner but was less effective, with 60% inhibition at 250 μg/mL ($P = .01$), and sLeβ inhibition reached a plateau of $\sim 40$% at 100 μg/mL ($P < .05$) (Figure 3B). Leβ(tet)PL was less effective, with 30% inhibition at concentrations $> 10$ μg/mL ($P < .05$). Control solutions of carriers (HSA and PL) only did not inhibit.

Combinations of inhibitors were also analyzed. Addition of sLeβ to Leβ(hex)PL or to Leβ-HSA increased inhibition to 96% at 10 μg/mL of each inhibitor and 93% at 50μg/mL of each inhibitor ($P < .001$), respectively (Figure 3C and 3D). Combination of sLeβ with domain antibody 25 increased the inhibition of binding from 20% to 40% at 10μg/mL (each inhibitor) and from 35% to 60% at 50 μg/mL ($P < .05$). At the higher concentration of 100 μg/mL, there was little increased effect on inhibition of binding, which was, in any case, almost maximal (Figure 3E).

Displacement of adherent H. pylori from stomach sections. The most potent glycoconjugate inhibitors were tested for their ability to displace previously bound H. pylori from human stomach sections. Ten micrograms per milliliter of Leβ(hex)PL removed 71.5%, and 250 μg/mL removed 69.2% of bound H. pylori, compared with the unconjugated polylysine (0% removal; $P = .001$) (Figure 5). Leβ-HSA displaced H. pylori with an incremental dose response, removing 35% of H. pylori at 10 μg/mL ($P < .05$).

DISCUSSION

Passive immunotherapy is a growing global market, with an estimated profit of $34 billion by 2010, and although most of these therapies are targeted at malignancies, some are anti-infective. The first commercial anti-infective monoclonal against respiratory syncytial virus had sales of $1.1 billion. Other intended targets are human immunodeficiency virus type 1, Mycobacterium tuberculosis, Clostridium difficile, Bacillus anthracis,
Figure 2. Surface plasmon resonance screening and analyses of inhibitors of BabA binding to Lewis\(^a\) antigen (Le\(^b\))–human serum albumin (HSA). Le\(^b\)-HSA was immobilized on the sensorchip surface, and binding of fluid-phase BabA or intact *Helicobacter pylori* was measured in the presence of inhibitors. 

**a**, Screening of 42 domain antibodies (dAbs) with binding activity to BabA. Binding is shown as a percentage relative to binding of fluid-phase BabA alone. Three dAbs (bold) completely inhibited BabA binding. dAb HEL4 of irrelevant specificity (hen egg lysozyme) was a control. 

**b**, Dose response of dAbs 25, 26, and 28 coinjected at varying concentrations with fluid-phase BabA (5 nmol/L). 

**c**, Effect of dAb 25 on binding of intact cells of 3 strains of *H. pylori*. Intact *H. pylori* (5 × 10\(^14\) colony-forming units/mL) was injected over immobilized Le\(^b\)-HSA and dAb 25 (1 μmol/L). 

**d**, Effect of poly-D-lysine conjugates of Le\(^b\) hexasaccharide (Le\(^b\)[hex]PL) on BabA binding. Superimposed sensorgrams (duplicates) are shown of BabA and Le\(^b\)[hex]PL binding to immobilized Le\(^b\)-HSA. 

**e**, Effect of poly-D-lysine conjugates of Le\(^b\) tetrasaccharide (Le\(^b\)[tet]PL) on BabA binding. Superimposed (duplicate) sensorgrams show inhibition of BabA binding to immobilized Le\(^b\)-HSA.
Candida albicans, Streptococcus pneumonia, and H. pylori (as detailed in this article) [19–23].

In the present study, we describe a human Vκ domain antibody that inhibits attachment of H. pylori to human stomach epithelium. Forty-three independent Vκ domain antibodies that bound BabA, isolated from the phage library, were screened for inhibition of BabA interaction with Leb with use of relatively fast-throughput binding assays based on SPR measurement of BabA binding to Leb-HSA. Domain antibodies 25 and 28 were tested for inhibition of H. pylori binding to human stomach epithelium, with only domain antibody 25 proving to be effective. Although preliminary SPR data suggest that domain antibody 25 (Kd, ∼1 × 10^{-7} mol/L) has lower affinity for BabA than does domain antibody 28 (Kd, ∼1 × 10^{-8} mol/L), epitope...
specificity may be a major factor that determines inhibitory activity. The epitope to which domain antibody 25 is directed has not been mapped, although it may overlap with the binding site of BabA for \( \text{Le}^b \). Binding studies with chimaeric BabA constructs indicate that the BabA binding site is within residues 212–614 [24].

Domain antibodies have potential for topical application to prevent infection with \( \text{H. pylori} \). In previous studies, domain antibodies that blocked adhesion mediated by the MP65 man-
jugates, which not only provide a means of oligomerising Leb and, hence, increase the avidity of the inhibitor, but also make use of a protease-resistant backbone to which Leb can be coupled. The Leb hexasaccharide conjugated to poly-o-lysine was as potent an inhibitor as Leb-HSA in molecular assays but was more potent as an inhibitor of attachment of H. pylori to human stomach epithelium. In addition, the Leb hexasaccharide conjugated to poly-o-lysine was 100-fold more effective than Leb-HSA in removing adherent H. pylori from human stomach tissue sections. Protease resistance of the poly-o-lysine backbone may be a significant factor in this increased efficacy. This demonstration that the inhibitors can displace adherent H. pylori is of great importance, because it suggests that, in addition to prophylactic treatment, topical application of adhesion-blocking compounds may have therapeutic potential for infected individuals. In contrast to the Leb glycoconjugates, domain-blocking compounds may have therapeutic potential for addition to prophylactic treatment, topical application of adhesion-blocking compounds may have therapeutic potential for infected individuals. In contrast to the Leb glycoconjugates, domain antibody 25 was ineffective at removing H. pylori when added after adherence of the bacteria, possibly reflecting the 2-log lower affinity for BabA, compared with Leb-HSA, which would again increase the desirability of multivalent presentation discussed above.

Use of combinations of inhibitors (sLe x with Leb conjugates or with domain antibody) may be a more effective strategy in preventing attachment of H. pylori. At low concentrations of each inhibitor (10 µg/mL), the inhibitory effects were synergistic, achieving almost complete inhibition, although at high concentrations (100 µg/mL), there was little increased effect on the inhibition.

An attractive future development might be to incorporate numbers of the active groups of these potentially synergistic partners on individual gold nanoparticles [26] or micelles. By presenting a high concentration of the inhibitors on the nanoparticles to the microbial surface, the effect may be to enhance inhibition of binding. Alternatively, the inhibitors may be linked to gold + iron nanoparticles and, once specifically bound to the organism, may be exposed to a variable magnetic flux, thereby rapidly heating the nanoparticles and killing the microorganism, irrespective of its antibiotic sensitivity.

References