Inhibition of *Helicobacter pylori* and *Helicobacter mustelae* Binding to Lipid Receptors by Bovine Colostrum

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Helicobacter pylori, **the etiologic agent of chronic-active gastritis and duodenal ulcers in humans, and *Helicobacter mustelae,* a gastric pathogen in ferrets, bind to phosphatidylethanolamine (PE), a constituent of host gastric mucosal cells, and to gangliotetraosylceramide (Gg4) and gangliotriaosylceramide (Gg3). The effect of a bovine colostrum concentrate (BCC) on the interaction of *H. pylori* and *H. mustelae* to their lipid receptors was examined. BCC blocked attachment of both species to Gg4, Gg3, and PE. Partial inhibition of binding was observed with native bovine and human colostra. BCC lacked detectable antibodies (by immunoblotting) to *H. pylori* surface proteins (adhesins). However, colostral lipid extracts contained PE and lyso-PE that bound *H. pylori* in vitro. These results indicate that colostrum can block the binding of *Helicobacter* species to select lipids and that binding inhibition is conferred, in part, by colostral PE or PE derivatives. Colostral lipids may modulate the interaction of *H. pylori* and other adhesin-expressing pathogens with their target tissues.**

Colostrum conveys protection to the immunologically naive offspring of many mammalian species against a variety of microbial pathogens by immunoglobulins and nonimmunoglobulin compounds [1]. Breast-feeding during the first months of life decreases human infant morbidity and mortality from diarrheal and systemic infections [1]. Whole bovine colostrum and immunoglobulin-enriched colostrum fractions have been used in infants and immunocompromised adults for the treatment of or prevention of enteric infections by bacterial, viral, and protozoal pathogens [2–5]. While secretory IgA is the major immunoglobulin of human colostrum and breast milk [1], the largest immunoglobulin fraction of bovine colostrum is IgG of the IgG1 subclass [6, 7].

Helicobacter pylori is the primary etiologic agent of chronic-active gastritis in children and adults [8–10]. *H. pylori* infection is the cause of nearly all primary duodenal ulcer disease, and chronic infection has been linked to gastric adenocarcinoma and mucosa-associated lymphoid tissue B cell lymphoma of the stomach [11, 12]. More than 50% of the adult population has serologic evidence of *H. pylori* infection by the age of 60 [13]. The prevalence of *H. pylori* infections is increased among children from socioeconomically disadvantaged families [14, 15] and in developing countries, where 80% of the population is infected by 20 years of age [14, 16, 17]. Epidemiologic studies from regions with a high prevalence of *Helicobacter* infections indicate that breast-feeding may be protective [18].

Adherence of *H. pylori* to the gastric mucosa is a critical step in the pathogenesis of the disease [19, 20]. Additional factors, such as expression of cytotoxin(s) and urease, and host inflammatory mediators are necessary to cause gastric or duodenal lesions [21, 22]. *Helicobacter mustelae* infection of ferret antrum mimics human *Helicobacter*–associated diseases and includes gastritis, duodenal ulcers, and gastric carcinoma [23, 24]. *H. pylori* and *H. mustelae* adhere closely to the host surface epithelium in vivo [23, 25] and human gastric epithelial and HEp-2 cells in culture [19, 26, 27]. Both *Helicobacter* species bind in vitro to a phospholipid, phosphatidylethanolamine (PE), and to the neutral glycolipids gangliotetraosylceramide (Gg4 or asialo-GM_1; Galβ1-3GalNAcβ1-4Galβ1-4Glu cer) and gangliotriaosylceramide (Gg3 or asialo-GM_2; GalNAcβ1-4Galβ1-4Glu cer) [28]. Quantitative adherence studies of *H. pylori* using various epithelial cell lines correlated the amount of PE with the efficiency of bacterial attachment [29]. *H. pylori* possess a PE-binding adhesin [30] that also recognizes Gg4 and Gg3 and competitively blocks the attachment of *H. pylori* and *H. mustelae* to these receptors [28, 30]. However, the molecular identity of the Helicobacter adhesin(s) is still elusive [20, 31].

The present study was designed to examine the effect of human and bovine colostrum on the interaction of *H. pylori* and *H. mustelae* with purified lipid receptors in vitro. We analyzed colostrum concentrate for the presence of antibodies to the PE-binding *H. pylori* envelope proteins (adhesin) and examined

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the interaction of *H. pylori* with fractionated colostral lipid extracts to gain insight into the mechanism(s) underlying the activity of colostrum.

Methods

Bacterial strains and growth conditions. *H. pylori* strain LC-11 [32], which was recovered from an antral biopsy of a child with gastritis and a duodenal ulcer, and *H. mustelae* strain SC [28], which was originally isolated from a ferret (provided by Steven E. Czin, Rainbow Babies and Children's Hospital, Cleveland), were stored as described [33] at −70°C in Brucella broth containing 10% fetal calf serum and glycerol. For binding assays, bacteria were grown in Brucella broth (Gibco Laboratories, New York) containing trimethoprim (5 mg/L), vancomycin (10 mg/L) (both from Sigma, St. Louis), and 10% fetal calf serum (Bockneck, Rexdale, Canada) or on solid blood agar at 37°C under reduced oxygen [28, 32].

Colostra and lipid compounds. Bovine colostrum was collected from healthy cows within 48 h of calving, pooled, and processed as described [7]. The preparation process removed most of the cholesterol, casein, and lipoproteins. The final lyophilized product, termed bovine colostrum concentrate (BCC; KMP Bio-Test Pharma, Dreieich, Germany), contained ~80% (dry weight) protein, of which 65% was immunoglobulins (predominantly IgG), <5% lactose, and <1.25% lipids [7] (unpublished data). Native bovine colostrum was provided by Stanley Read (Hospital for Sick Children, Toronto). Human colostrum was from excess samples that had been collected within 10 days postpartum (courtesy of Debbie Stone, Hospital for Sick Children). Native bovine and human colostra were kept frozen at −20°C, and were thoroughly mixed and sonicated prior to use. *Escherichia coli* L-α-PE and Gg4 were from Sigma (St. Louis) and Matreya (Pleasant Gap, PA), respectively. Gg3 from bovine brain was prepared in this laboratory as described [28].

Bacterial overlay binding assay. Bacterial binding to lipid species was monitored as described [32]. In brief, indicated amounts of purified PE, Gg3, and Gg4 were separated by thin-layer chromatography (TLC) in chloroform-methanol-water (65:25:4, by volume), using 0.25-mm silica gel plastic sheets (Polygram Sil G; Macherey-Nagel, Düren, Germany). Replicate TLC plates were dried and stained with orcinol or iodine (controls) or blocked with 3% gelatin in H_2O for 2 h at 37°C and washed three times with distilled water. Blocked plates were then incubated for 2 h at 37°C under microaerobic conditions with a fresh overnight culture of motile *H. pylori* or *H. mustelae* suspended in 20 mL of Brucella broth (10^7 cfu/mL). After being washed five times with 0.1 *M* Tris-buffered saline (TBS, pH 7.6) to remove nonadherent bacteria, TLC plates were incubated with a 1:500 dilution of rabbit anti-*Helicobacter* flagella antibody [28, 32] in TBS containing 1% bovine serum albumin (Sigma) for 18 h at 4°C. Plates were washed in TBS, and bound bacteria were visualized, using a 1:2000 dilution of goat anti-rabbit horseradish peroxidase (HRP) conjugate antibody (Bio-Rad, Richmond, CA) and 4-chloro-1-naphthol (Sigma) as substrate.

Bacterial binding inhibition assay. Overnight bacterial cultures were diluted in Brucella broth containing various concentrations (5–200 mg/mL) of the lyophilized BCC or native colostrum, as indicated. The mixture was incubated for 1 h at 37°C under microaerobic conditions on a rotator prior to the overlay procedure. Bound bacteria were detected as described above. In control experiments, TLC plates were incubated with BCC for 1 h at 37°C and washed with TBS prior to the bacterial overlay. BCC did not interfere with the binding of the antiflagella antibody to *H. pylori* or *H. mustelae* (see figures 1 and 6).

Immunoblot analysis. Crude *H. pylori* adhesin (PE-binding material) was extracted from fresh bacterial cultures as described [30, 34]. In brief, *H. pylori* was grown under microaerobic conditions at 37°C, suspended in distilled water, and vortexed. The water soluble supernatant (water extract) was size-fractionated by SDS polyacrylamide electrophoresis (7.5% acrylamide) and stained with Coomassie blue or electroblotted to a nitrocellulose membrane (Protran; Schleicher & Schuell, Keene, NH). Blotted membranes were blocked with 0.25% gelatin and 2% bovine serum albumin in 50 m*M* TBS-0.1% Tween and incubated overnight with serial dilutions of BCC or a mouse polyclonal antibody to column-purified, PE-binding surface protein (anti–*H. pylori* adhesin) at a 1:200 dilution (gift from G. Cates, Connaught, North York, Ontario). Bound bovine or mouse antibodies were detected using rabbit anti-bovine IgG (Sigma; diluted 1:20000) or goat anti-mouse IgG–HRP conjugate antibody (Bio-Rad; diluted 1:10,000) with the Enhanced Chemiluminescence technique (Amersham Life Science, Arlington Heights, IL). Rabbit anti-bovine-HRP conjugate antibody was previously shown, using a dot-blot immunoassay, to recognize bovine colostral IgG (unpublished data). All antibodies were diluted in 50 m*M* TBS–0.1% Tween 20 with 1% (wt/vol) bovine serum albumin.

Colostral lipid extraction and analysis. Lipids were extracted from colostrum, using the Folch procedure [35]. In brief, a 100-mg/mL solution of BCC in distilled H_2O or native colostrum was thoroughly mixed, sonicated, extracted overnight with 18 vol of chloroform-methanol (2:1, by volume) with stirring, filtered through Whatman paper, and partitioned against water in the presence of KCl (final ratio of chloroform–methanol–KCl 0.88%, 2:1:0.6 by volume). The dried lower-phase lipids were applied to a silicic acid column (Rose Scientific, Edmonton, Canada), which had been equilibrated and eluted with chloroform, followed by acetone-methanol (9:1, by volume) and methanol. The eluted fractions were dried under N_2, weighed, redissolved in chloroform-methanol (2:1), and stored in sealed containers at −70°C. Human red blood cells were extracted with 11 vol of 2-propanol and 7 vol of chloroform and then separated on a silica column.

Lipid extracts were fractionated by TLC as described above. Dried TLC plates were stained with iodine or molybdenum blue for the detection of lipids and phospholipids, respectively, or with orcinol for the detection of glycolipids [32]. Characterization of colostrum-derived phospholipid species was sought by TLC immunostaining with a monospecific rabbit anti-PE antibody (from this laboratory). In brief, TLC plates were blocked as described above and incubated with a 1/20 antibody dilution. Bound anti-PE was visualized using goat anti-rabbit IgG and the peroxidase detection system.

Results

Binding of Helicobacter species to lipid receptors. As reported previously [28], *H. pylori* and *H. mustelae* bound Gg4, Gg3, and PE in the TLC overlay assay. Both *Helicobacter*

species also bound to deacylated (lyso-) PE, a PE-derived lipid species present in most PE preparations (figure 1 A–C).

Binding inhibition by colostrum. Preincubation of *H. pylori* and *H. mustelae* with BCC at a concentration of 100 mg/mL inhibited binding of both *Helicobacter* species to Gg4 and Gg3 (figure 1D, E). Incubation with BCC, compared with controls, did not reduce the number of viable bacteria (colony-forming units; data not shown). When colostrum-treated bacteria were washed prior to the overlay assay, complete binding inhibition persisted. However, when the TLC plates were preincubated with BCC and washed prior to the addition of untreated bacteria, binding of *Helicobacter* species to Gg4, Gg3, and PE was not affected (results not shown). This indicates that inhibition of bacterial binding was not conferred by the blockade of solid-phase lipid receptors but by direct interaction of the bacteria with colostrum.

BCC-mediated inhibition of *Helicobacter* species binding was monitored over 5–200 mg/mL. BCC completely blocked attachment of *H. pylori* to Gg4 and Gg3 at 100 mg/mL and to PE at 200 mg/mL (figure 2). Binding of both *Helicobacter* species to lyso-PE was abolished at 5 mg/mL.

Native colostrum blocked attachment of *H. pylori* less effectively than did BCC. At the concentrations used (35%–40%, vol/vol), native bovine and human colostrum consistently caused a moderate decrease in binding to Gg4 and PE (figures 2E and 3C). In contrast, native bovine colostrum completely inhibited binding to Gg3 (human colostrum not examined), and both native colostra completely inhibited binding to lyso-PE (figures 2 and 3).

Immunoblot analysis for colostral adhesin antibodies. The mouse anti–*H. pylori* adhesin antibody recognized two bands of ~63 and 50 kDa under denaturing and reducing conditions of similar intensity (figure 4, lane 1), confirming the presence of PE-binding proteins in the water soluble bacterial extract. Serial dilutions of BCC (1–0.02 mg/mL, dry weight) failed to produce a specific signal, indicating that bovine colostrum lacks detectable antibodies to PE-binding surface proteins of *H. pylori* (figure 4, lanes 3 and 4). Anti-bovine HRP-conjugate antibody was previously shown to react with BCC.

Analysis of colostrum-derived lipid compounds. To explore the possibility that colostrum contained lipid or glycolipid compounds, which compete with bacterial ligand(s) for their lipid receptors, BCC and native colostrum samples were extracted by Folch partition. The acetone-methanol and the methanol fractions of the organic (lower) phase were separated by TLC (figure 5). Neutral glycolipids corresponding to Gg3 or Gg4 were not detected with this approach (figure 5A, acetone-methanol fractions, lanes 3–6). In two separate BCC extracts, an orcinol and iodine staining band was observed at the origin of the thin-layer chromatogram, suggesting the presence of a highly polar glycolipid (figure 5A, C, lane 4). Molybdenum blue staining of the chromatogram of the methanol fractions of all colostra revealed three major bands, indicating colostrum-derived phospholipids (figures 5B and 6A). The positions of the upper and middle band corresponded to control PE and lyso-PE from *E. coli* and human red blood cells (figures 5B, C and 6A, lanes 1 and 2). Both lipid species reacted with anti-PE (figure 5D). The yield of the dried, phospholipid-containing methanol fraction was ~1.2 mg/g BCC and 0.45 and 0.18 mg/mL of native bovine and human colostrum, respectively.

We hypothesized that colostrum-derived phospholipids or hitherto undetected lipid compounds might play a role in the

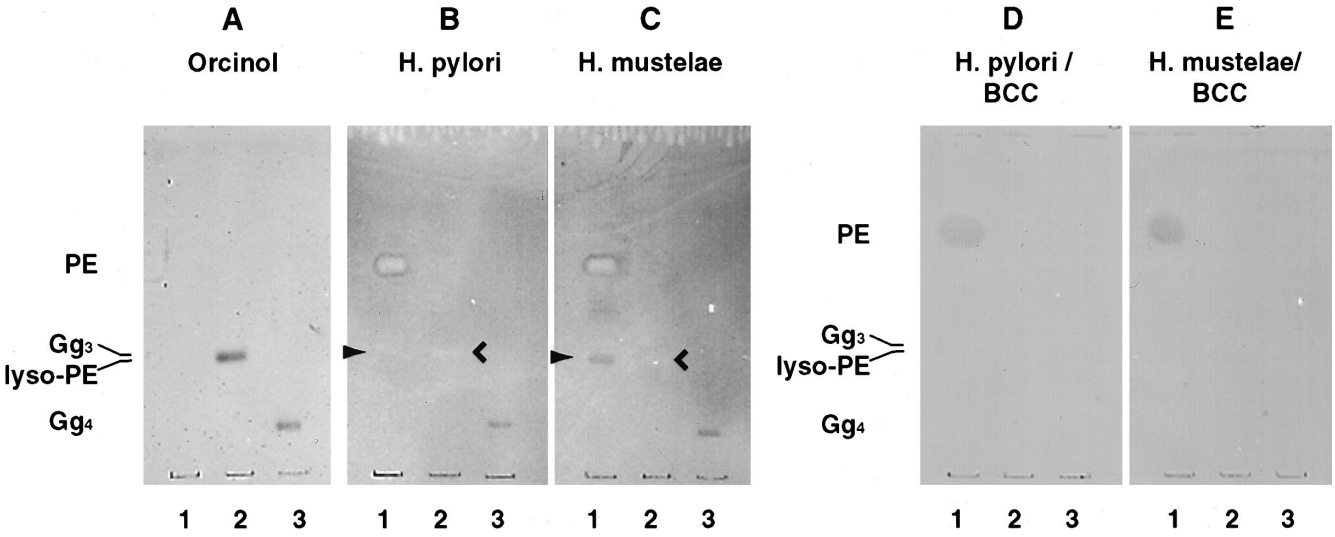

Figure 1. Inhibition of *H. pylori* and *H. mustelae* binding to lipid receptors by bovine colostrum concentrate (BCC; 100 mg/mL) detected by thin-layer chromatogram (TLC) overlay procedure. **A**, TLC stained with orcinol. **B**, *H. pylori* overlay. **C**, *H. mustelae* overlay. **D**, *H. pylori* incubated with BCC. **E**, *H. mustelae* incubated with BCC. Lanes: 1, phosphatidylethanolamine (PE; 30 μg); 2, Gg3 (asialo-G_{M2}, 4 μg); 3, Gg4 (asialo-G_{M1}; 4 μg); solvent was chloroform-methanol-water (65:25:4, by volume). In **B** and **C**, ''negative'' staining (PE, occasionally Gg3) represents bacterial binding [30] and is likely due to ''prozone'' phenomenon. Arrowheads denote positions of Gg3 (open) and lyso-PE (solid).

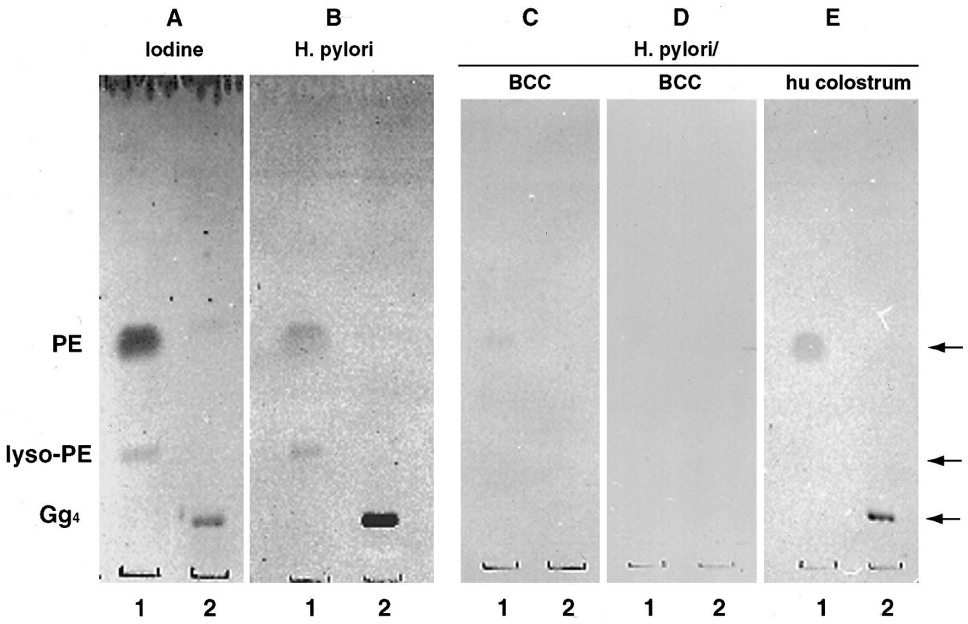

Figure 2. Effect of bovine and human colostrum on attachment of *H. pylori* to lipid receptors (thin-layer chromatography overlay assay). **A**, staining of separated lipids with iodine. **B**, *H. pylori* overlay (control). **C**, preincubation of *H. pylori* with bovine colostrum concentrate (BCC) 100 mg/mL. **D**, BCC 200 mg/mL. **E**, human colostrum 40% (vol/vol). Lanes: 1, PE (phosphatidylethanolamine; 30 μg); 2, Gg4 (asialo-GM_1; 4 μg). Arrowheads depict partial blocking of bacterial binding to PE, lyso-PE, and Gg4, as shown.

observed inhibition of *Helicobacter* binding to PE, Gg4, and Gg3 in vitro. Therefore colostral lipid extracts were separated by TLC, and the TLC plates were assayed for bacterial binding. *H. pylori* adhered to the upper and middle colostrum-derived phospholipid bands (figure 6B). In addition, binding to an orcinol-sensitive, strongly polar band in the BCC extract that did not migrate from the origin was observed (figure 6B, C, lane 3). Incubation with BCC (200 mg/mL) prevented binding of *H. pylori* to two phospholipid species corresponding to PE and lyso-PE, respectively, but not to the highly polar compound (figure 6C, lane 3). Binding to the middle phospholipid band comigrating with lyso-PE was also blocked by native bovine and human colostrum (results not shown). These findings indicate that *H. pylori* interacts with colostrum-derived PE and lyso-PE. Thus, the observed blockade of *Helicobacter* adhesion to their lipid receptors could be mediated by colostral PE.

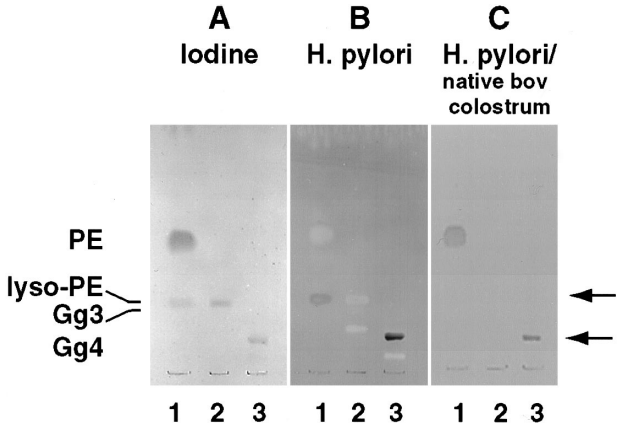

Figure 3. Effect of native bovine (bov) colostrum on *H. pylori* binding to phosphatidylethanolamine (PE), Gg3, and Gg4. **A**, iodine staining; B, *H. pylori* binding (control); C, pre-incubation of *H. pylori* with native bovine colostrum, 36% (vol/vol). Lanes: 1, PE (40 μg); 2, Gg3 (asialo-G_{M2}; 6 μg); and 3, Gg4 (asialo-GM_1; 4 μg). Arrows on right indicate (partial) inhibition of bacterial binding to lyso-PE, Gg3, and Gg4, as shown.

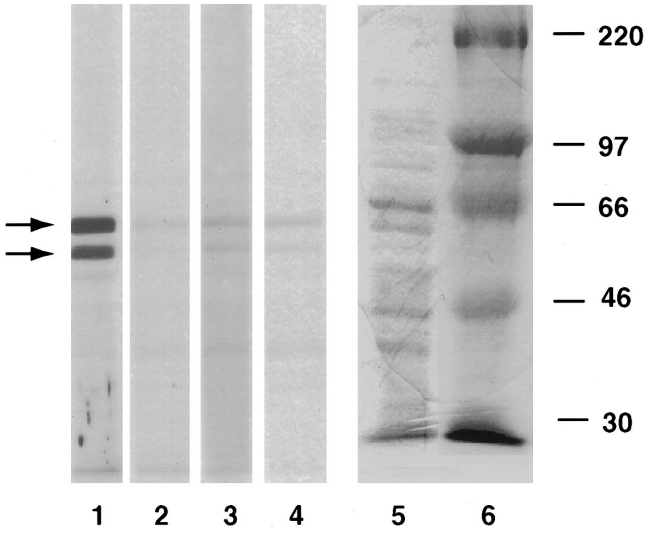

Figure 4. Western blot analysis to detect antibody to *H. pylori* adhesin/surface proteins in bovine colostrum. *H. pylori* water extract (2 μg protein/lane) was size-fractionated by SDS-PAGE (7.5% acrylamide) and blotted to nitrocellulose (lanes 1–4), or stained with Coomassie blue (lanes 5 and 6). Lane 1, blot probed with mouse antibody to phosphatidylethanolamine-binding *H. pylori* surface protein (adhesin; 1/200 dilution); 2, control without primary mouse antibody; 3, blot probed with bovine colostrum concentrate (BCC; 0.1 mg/mL); 4, control without BCC; 5, Coomassie blue–stained gel; 6, molecular size markers. Mouse antibody recognizes 2 discrete bands of ~63 and 50 kDa under reducing and denaturing conditions (arrows, lane 1).

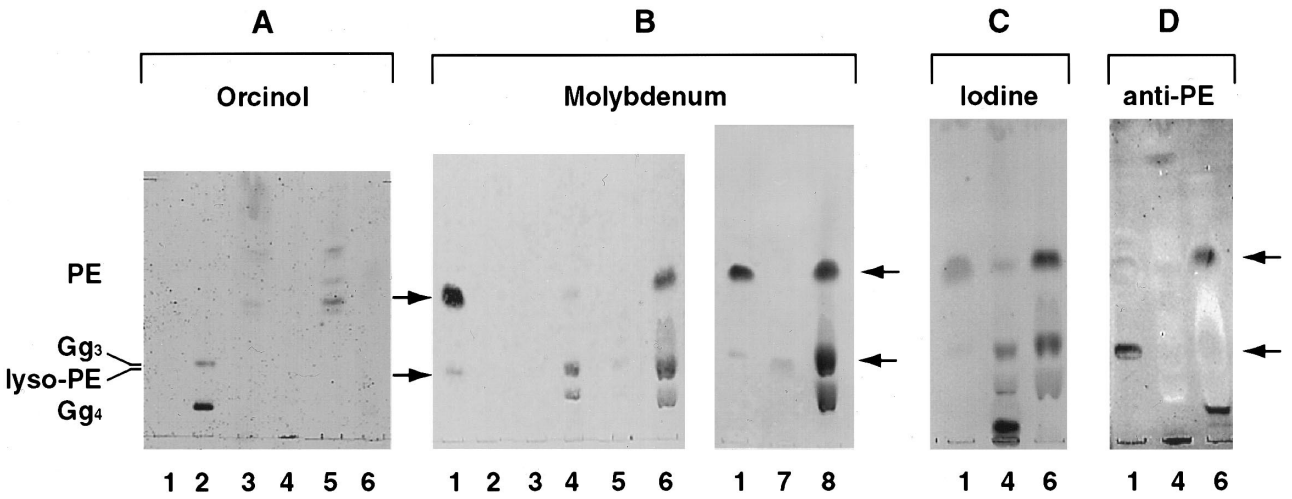

Figure 5. Thin-layer chromatogram of lipid fractions of bovine colostrum concentrate (BCC) and native bovine colostrum. **A**, chromatogram stained with orcinol. **B**, molybdenum blue. **C**, iodine. **D**, immunostaining with rabbit anti-phosphatidylethanolamine (PE) antibody. Lanes: 1, PE (30 μg); 2, Gg3 and Gg4 (both 5 μg); 3, BCC (extraction A, acetone-methanol fraction; 75 μg); 4, BCC, (extraction A, methanol fraction; 75 μg); 5, native colostrum (acetone-methanol fraction; 75 μg); 6, native colostrum (methanol fraction; 100 μg); 7, human colostrum (acetone-methanol fraction; 100 μg); 8, human colostrum (methanol fraction; 100 μg). Arrows indicate PE and lyso-PE, respectively. Gg4, asialo-GM_1.

Discussion

H. pylori and *H. mustelae* adhesion to host antral epithelial cells is required to initiate and maintain infection resulting in chronic gastric disease [23, 25, 36]. In the present study, we showed that bovine colostrum blocked the attachment of *H. pylori* and *H. mustelae* to immobilized receptors, the glycolipids Gg4 and Gg3 and PE. The interaction of *H. pylori* to these structurally diverse lipid receptors appears to be mediated by a PE-binding, binary adhesin [30, 37]. This *H. pylori* adhesin, which is constitutively expressed on the bacterial surface and recovered from water soluble extracts, was previously shown to bind PE, Gg4, and Gg3 in vitro [30]. Our results indicate that constituents of bovine colostrum can block *Helicobacter* adhesin(s) that facilitate interaction with these lipid receptors. Both *H. pylori* and *H. mustelae* recognize parent PE and deacylated (lyso-) PE with similar efficiency [28]. Lyso-PE is formed by phospholipase A_2-catalyzed hydrolysis of a fatty acid residue in β-position at the glycerol backbone. *Helicobacter* binding to lyso-PE, present in commercial PE preparations as well as in lipids extracted from tissue culture cell lines [28, 29], was blocked by BCC at much lower concentrations than was the binding to PE and Gg4. Furthermore, concentrations of native colostra that were unable to effectively block *Helicobacter* binding to PE or Gg4 clearly inhibited binding of the gastric pathogen to lyso-PE.

The contribution of the PE, lyso-PE, Gg4, Gg3, and other putative receptors for *H. pylori* to successful microbe-cell interaction is not yet fully understood [20, 38]. As with other human pathogens, it has been proposed that *Helicobacter* species express multiple adhesins that would allow bacterial interaction with various target cell epitopes and mucin components, including sulfatides and sialylated or fucosylated glycoconjugates [39–44]. Adhesin expression is likely influenced by environmental stimuli, such as strongly acidic conditions. For example, a heat (and acid) shock–inducible surface protein of 70 kDa has been recently described that mediates the attachment of *H. pylori* to sulfogalactosylceramide [37].

Secreted immunoglobulins have been implicated in many of the reported antimicrobial effects of colostrum [1]. For example, high IgA antibody titers to *H. pylori* in breast milk were shown to correlate with a reduced incidence of *H. pylori* infection in breast-fed infants from The Gambia [18]. The authors did not characterize the antigenic epitope(s) that were recognized by colostral IgA. We were unable, by immunoblotting, to detect bovine colostral antibodies against water soluble proteins from *H. pylori*, including the PE-binding fraction. Korhonen et al. [45], using *H. pylori* glycine extracts in an EIA, also failed to detect *H. pylori*–specific antibodies in nonimmune bovine colostrum.

The antimicrobial activity of milk and colostrum has been related, in part, to their content of glycoproteins and glycolipids, which competitively block the adherence of infectious agents or toxins to their cognate host cell membrane receptors [1, 46, 47]. In this study, we demonstrated that BCC and native bovine and human colostra contained substantial amounts of extractable phospholipids, including PE and lyso-PE (figure 5B). PE is a major constituent of the phospholipid fraction of colostrum. Phospholipids account for 0.5%–1% of total fat in human breast milk (0.2–0.4 mg/mL), 20%–30% of which is PE [48, 49]. The source of most colostral PE in human and bovine colostrum is the plasma membrane that envelops the milk fat globules [49]. Data from Isaacs and Thormar [47] and others [48] show that lipase activity is needed to initiate antimicrobial activity of breast milk in vivo. Lipases secreted from the chief cells of the gastric mucosa appear to be particu-

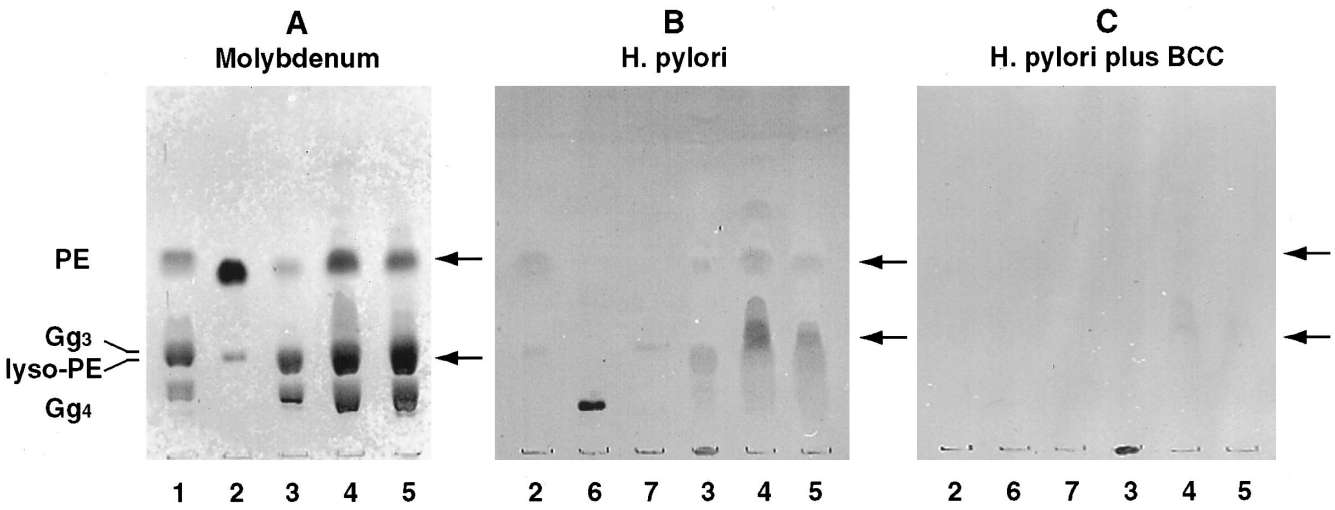

Figure 6. Analysis of bovine colostrum–derived lipid extracts. **A**, Thin-layer chromatogram (TLC) stained with molybdenum blue. **B**, TLC overlay with *H. pylori.* **C**, Inhibition of *H. pylori* binding to colostrum-derived lipids by unfractionated bovine colostrum concentrate (BCC; 200 mg/L). Lane 1, human erythrocyte extract (methanol extract; 60 μg); 2, phosphatidylethanolamine (PE) and lyso-PE (30 μg); 3, BCC (extraction B, methanol fraction; 90 μg); 4, native bovine colostrum (methanol fraction; 100 μg); 5, BCC (extraction A, methanol fraction; 100 μg); 6, Gg4 (asialo-G_{M1}; 4 μg); 7, Gg3 (asialo-G_{M2}; 6 μg). Arrows indicate phospholipid species that correspond to PE and lyso-PE.

larly effective [48]. It is possible that the interaction of colostrum-derived PE and lyso-PE with the *Helicobacter* adhesin blocks binding to Gg4 and Gg3. Clyne et al. [50] recently reported that whole human milk reduced adherence of *H. pylori* to gastric adenocarcinoma (Kato III) cells and that this effect was unrelated to the presence of milk (and serum) immunoglobulins to *H. pylori*. This finding perfectly fits with our observation.

While detectable amounts of Gg4 or Gg3 were not present in the colostral lipid extracts, we observed a highly polar compound, likely polyglycosylceramide(s), which bound *H pylori.* Complex carbohydrates, such as Lewis blood group antigens or fetuin can bind *H. pylori* under certain conditions [39, 40, 51]. However, bacterial attachment to this compound was not reduced by the parent colostrum. Strömqvist et al. [52] recently identified a fucosylated colostral protein, κ-casein, which inhibited *H. pylori* adhesion to gastric mucosa, probably by interfering with Lewis (Le^b) blood group–related receptors. Of interest, the human but not the bovine κ-casein variety was an effective inhibitor, emphasizing the diversity of *Helicobacter* adhesins and host-specific receptors.

We showed that bovine colostrum has potential anti-adhesive activity for both *H. pylori* and *H. mustelae.* The findings demonstrate that colostrum-derived factor(s) can block the interaction of these gastric pathogens with selected lipids in vitro. It is important to note, however, that multiple, constitutive or inducible adhesins and receptors are involved in *Helicobacter*–host cell interactions [19, 20, 37]. Prevention of bacterial adherence in vivo is complex and may not be achieved by targeting a single ligand or receptor. Studies are now needed to test the ability of various colostrum preparations to prevent the adherence of *Helicobacter* species in vivo, for example using the model of ferret gastritis. The identification of PE and lyso-PE in colostrum extracts not only directs attention to these lipid compounds as receptor analogues with potential antiinfective properties, but it also provides a tool to further dissect bacterial interactions with their host cell receptors.

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