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The Glycerolipid Receptor for *Helicobacter pylori* (and Exoenzyme S) Is Phosphatidylethanolamine

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We have previously shown that *Helicobacter pylori* specifically binds to a glycerolipid species preferentially found in the antrum of the human stomach. We now show by high-pressure liquid chromatographic analysis that this species is a form of phosphatidylethanolamine and that *H. pylori* specifically binds to bona fide phosphatidylethanolamine as detected by a thin-layer chromatogram overlay procedure. Considerable variation in the binding of *H. pylori* to phosphatidylethanolamine from different sources was observed, however, suggesting the importance of the nature of the long-chain hydrophobic moiety. A similar binding specificity was shown by exoenzyme S from *Pseudomonas aeruginosa*, consistent with our hypothesis that that an exoenzyme S-like adhesin is responsible for the binding of *H. pylori* to its lipid receptors.

Colonization of the human stomach with *Helicobacter pylori* has been implicated as the major etiological agent in the development of gastritis and possibly subsequent duodenal ulcer (3, 6, 27) and gastric carcinoma (17, 19, 20). Because of the known tropism of this organism for colonization of gastric epithelium, even when mislocated elsewhere in the gastrointestinal tract (31), we previously investigated the presence of gastric membrane species able to specifically bind to this organism, as monitored by the solid-phase thin-layer chromatography (TLC) overlay procedure (13). We isolated a charged glycerolipid species from the antrum of human stomach and from human erythrocytes which was specifically recognized by this organism, using this procedure.

Exoenzyme S is an ADP-ribosyltransferase and is one of the virulence factors of *Pseudomonas aeruginosa* involved in the pathogenesis of this organism (30). We have recently found (14) that this enzyme also specifically binds to the *H. pylori* receptor glycerolipid.

We now report on the partial structural characterization of this receptor species.

MATERIALS AND METHODS

Phosphatidylethanolamine (PE) from various tissues; phosphatidic acid, phosphatidylserine, and phosphatidylcholine from egg yolk; and phosphatidylglycerol from bovine brain were purchased from Sigma. Plastic-backed silica gel (SIL G) TLC plates were from Brinkman Inc. Goat anti-rabbit–horseradish peroxidase conjugate was from Bio-Rad.

Purification of *H. pylori* receptor. The *Helicobacter* glycerolid receptor was purified as previously described from pooled human erythrocytes (13). Briefly, the cells were extracted with chloroform-methanol (2:1, vol/vol), and lower-phase lipids were separated by silicic acid chromatography. The column was eluted in sequence with chloroform, acetone-methanol (3:1), and finally methanol. The methanol fraction was reapplied on a fresh column which was eluted with a linear polarity gradient of chloroform methanol (10:1 to 2:1).

Receptor binding. *H. pylori* was grown on Skirrow's medium and transferred to brucella broth supplemented with 10% fetal calf serum before binding (13). Only highly motile cultures were used for binding experiments. Purified exoenzyme S (29) and polyclonal rabbit antibody were a generous gift from D. Woods, University of Calgary. Exoenzyme S had been purified from *P. aeruginosa* grown in the presence of 5 mM EDTA, and receptor binding assays were performed in the presence of 5 mM $CaCl_2$ and 5 mM $MgCl_2$, which promotes the binding to the *Helicobacter* glycerolipid receptor (14). Exoenzyme S prepared from *P. aeruginosa* grown in the absence of EDTA is relatively unstable and therefore was not used in these studies.

The binding of *H. pylori* (13) and of exoenzyme S (12) to receptor lipids was monitored by TLC overlay as previously described. Essentially, pure lipid species (5 μg) were separated by TLC in chloroform-methanol-water (65:25:4, vol/vol/vol). The TLC was blocked with 2% gelatin at 37°C and incubated with freshly cultured *H. pylori* organisms (10^6/ml under microaerophilic conditions) or purified exoenzyme S (3 μg/ml of phosphate-buffered saline). After the washing step, bound organism or enzyme was detected by using anti-*H. pylori* rabbit antiserum (13) or exoenzyme S antiserum (14) and a peroxidase conjugate detection system (12).

Control incubations in the absence of *H. pylori* or exoenzyme S showed no binding in the overlay procedure.

HPLC analysis. The *Helicobacter* glycerolipid receptor was purified from human erythrocytes by silicic acid chromatography as described previously (13). The receptor was analyzed for phospholipid content by high-pressure liquid chromatography (HPLC) by the procedure of Heinz et al. (7). The molecular species of the glycerol moiety was determined after phospholipase C digestion, methanolysis, HPLC, and gas-liquid chromatographic separation and peak integration as described by Myher et al. (15).

RESULTS

Phospholipid analysis. Figure 1 shows the purification of the *H. pylori*-binding glycerolipid by silicic acid chromatography. The reactive species was subjected to analysis by HPLC in comparison with a mixture of human erythrocyte

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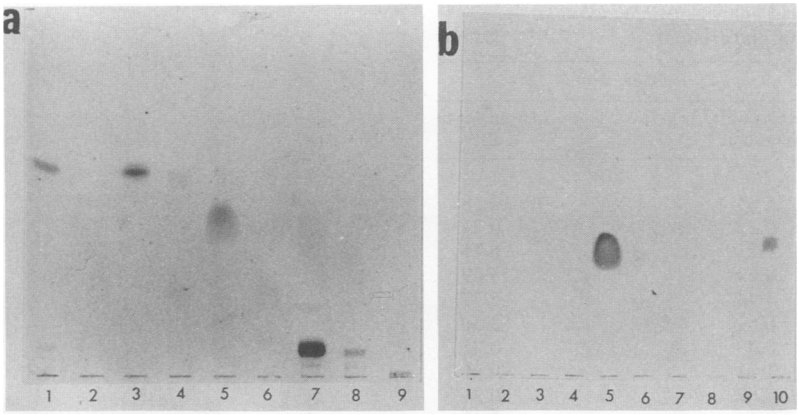

FIG. 1. Purification of *H. pylori* receptor from human erythrocytes. The *H. pylori* glycerolipid erythrocyte receptor was purified by gradient elution of a silicic acid column as described in Materials and Methods. Equal aliquots from different stages of the gradient were separated by TLC (chloroform-methanol-water, 65:/25:4, vol/vol/vol). (a) Visualized by orcinol. (b) *H. pylori* binding. Lanes 1 to 9, fractions eluted with solvent of increasing polarity; lane 10, aliquot of unfractionated extract.

phospholipids and purified erythrocyte PE isolated by TLC (15). The HPLC profiles are shown in Fig. 2. The *H. pylori* receptor was found to have a retention time identical to that of the human erythrocyte PE standard.

A comparison of the HPLC fatty acid analysis of the *H. pylori* receptor and erythrocyte PE after methanolysis is shown in Table 1. The fatty acid compositions were essentially identical.

Phospholipid binding. The binding of *H. pylori* to standard phospholipids was determined by TLC overlay (Fig. 3, panel 2). Only binding to PE was observed. Similar results were obtained for exoenzyme S (Fig. 3, panel 1), although additional low-level binding to phosphatidylinositol was also seen.

The ability of PE from various sources to bind *H. pylori* and exoenzyme S is shown in Fig. 4. The different PEs varied greatly in their ability to bind; however, the discrimination between the different PEs was the same for *H. pylori* and exoenzyme S. Although each species was of approximately equal purity (some impurities were detected in dog brain PE [Fig. 4a, lane 4]), PE from *Escherichia coli* was by far the most effective receptor (Fig. 4b and c). Bovine brain, porcine liver, egg yolk, and soybean PE were recognized, while PE from bovine liver and dog brain showed no receptor activity.

DISCUSSION

Current evidence strongly implicates gastric infection with *H. pylori* as an etiological agent in the development of human antral gastritis and duodenal ulcer and more recently as a risk factor for gastrointestinal carcinoma (17, 19, 20). We had purified lipid species from human erythrocytes, human and pig stomach, and cultured HEp2 cells (13) which were specifically bound by *H. pylori*. All these tissues have been shown to bind this bacterium. The receptor was more abundant in the stomach antrum as opposed to the fundus and was barely detectable in the antrum of children. This

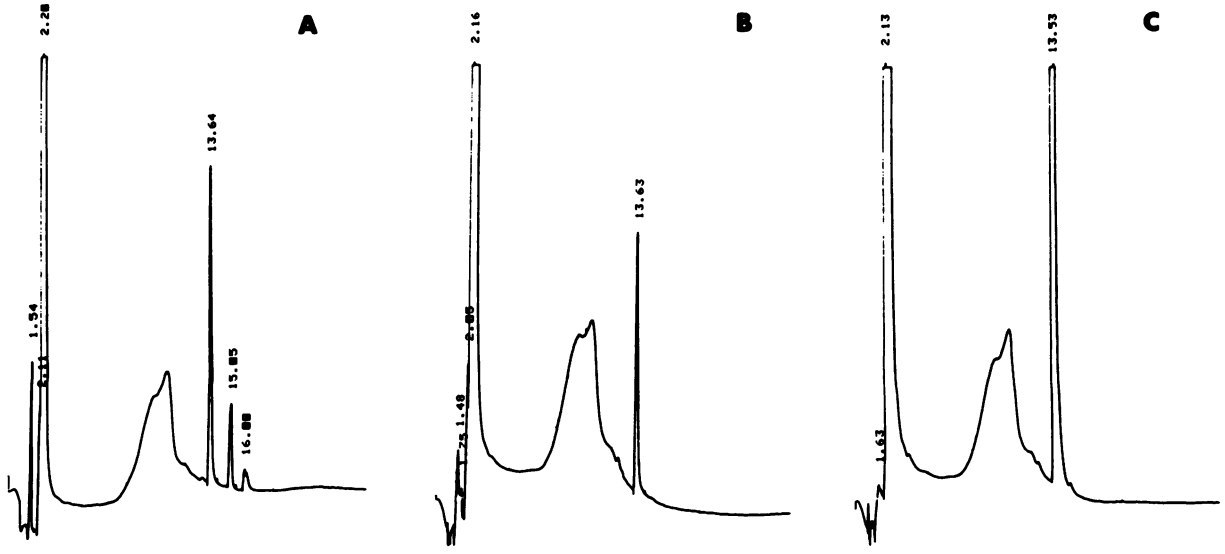

FIG. 2. HPLC analysis of *H. pylori* receptor. (A) Standard erythrocyte phospholipids. (B) Erythrocyte PE purified as described previously (15). (C) Purified *H. pylori* receptor from human erythrocytes (fraction 5 in Fig. 1).

TABLE 1. Fatty acid analysis of *Helicobacter* receptor glycerolipid

FAME + DMA[a]	Area (%)	
	Receptor glycerolipid[b]	Erythrocyte PE
FAME		
16:0	15.05	12.18
17:0	0.32	0.21
18:0	8.71	6.11
18:1	19.69	15.79
18:2w6	4.44	5.37
20:1w9	0.61	0.30
20:2w6	0.40	0.21
20:3w6	1.01	0.85
20:4w6	17.67	20.34
20:5w3	0.10	0.60
22:4w6	5.15	5.58
22:5w3	0.59	0.87
22:5w3	2.12	3.19
22:6w3	3.33	4.64
DMA		
16:0	5.05	6.33
17:0	0.50	0.91
18:0	11.01	11.85
18:1	4.24	4.66

[a] FAME + DMA, fatty acid methyl esters plus dimethyl actetals.
[b] From human erythrocytes.

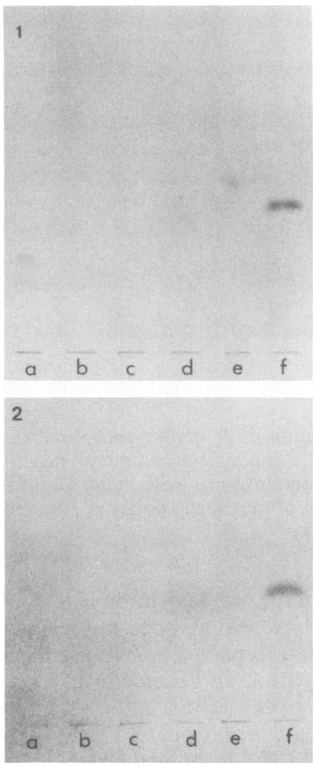

FIG. 3. Phospholipid binding specificity. (1) Binding of exoenzyme S; (2) binding of *H. pylori*. Individual phospholipids (5 μg) were separated by TLC and overlaid as described in Materials and Methods. Lanes: a, phosphatidylinositol; b, phosphatidylserine; c, phosphatidic acid; d, phosphatidylglycerol; e, phosphatidylcholine; f, PE.

correlates with the known colonization sites of this organism. The receptor was base sensitive, showing the loss of a single fatty acid, characteristic of an alkylacyl glycerolipid. The compound was weakly reactive with orcinol and was partially eluted in the glycolipid fraction when purified by silicic acid chromatography (13).

We have recently shown that exoenzyme S purified from *P. aeruginosa* also binds to the *H. pylori* receptor and to the glycolipids gangliotriaosyl and gangliotetraosyl ceramide (Gg_3 and Gg_4, respectively) (10). *H. pylori* also binds these ganglioseries glycolipids (data not shown). *Chlamydia* species also recognize PE and Gg_3/Gg_4 (11). In each of these cases, however, the role of Gg_3/Gg_4 binding in pathogenesis is uncertain since target tissues do not contain detectable levels of these glycolipids.

Other receptor-adhesin interactions have been reported for *H. pylori*. Binding of *H. pylori* to GM_3 ganglioside and sulfogalactosyl ceramide has been found (24). We also found low-level binding of GM_3 by *H. pylori* (12a) but have found no binding to sulfogalactosyl ceramide. However, PE is the major *H. pylori* receptor in the total lipid extract of human antrum. Sialyl lactose has been reported to be involved in the hemagglutination of erythrocytes by *H. pylori* (4), and this may relate to the recognition of GM_3. Indeed, GM_3 (and sulfolactosyl ceramide) inhibit *H. pylori* erythrocyte agglutination (25). Although treatment of erythrocytes with neuraminidase prevents erythrocyte agglutination (22), similar treatment of HEp2 cells does not prevent *H. pylori* adhesion (1). Moreover, in HeLa cells, binding studies are consistent with recognition of PE and not sialyl lactose (5).

Our present results show that both *H. pylori* and exoenzyme S specifically bind to PE and that this phospholipid represents the previously isolated *H. pylori* receptor glycerolipid (13). Our present studies only analyzed the receptor isolated from erythrocytes since PE from erythrocytes has been extensively characterized (Table 1) (15). The receptor species from human stomach is, we speculate, the same compound, but it may have a different fatty acid-ether composition.

It is possible that an exoenzyme S-related protein is responsible for the binding specificity of *H. pylori*. Exoenzyme S has recently been confirmed as an adhesin for *P. aeruginosa* (2). PE is a widely distributed phospholipid, and thus binding per se cannot explain the tissue tropism observed for *H. pylori* (31). Two points, however, are worthy of consideration. First, at least in erythrocytes, PE is predominantly a component of the inner leaflet of the plasma membrane bilayer (18, 32) and thus might not be available as a receptor for an extracellular ligand in most cells. Second, a wide disparity in the ability of PEs from different sources to bind *H. pylori* and exoenzyme S was observed (Fig. 4), suggesting that the lipid backbone plays a role in the possible receptor function of PE. In separate studies, we have reported that the lipid moiety of glycolipids can markedly influence their carbohydrate-mediated receptor function (21), and thus, this may present an as yet unappreciated mode of modulation of plasma membrane receptor activity.

Differences in the distribution of the *Helicobacter* receptor as monitored by TLC overlay were observed in the antrum compared with the fundus of the stomach and in juvenile opposed to adult antrum (13). Whether these differences represent different levels of PE or different components within the lipid moiety will be the subject of future study.

It is possible that binding to host PE itself plays a role in

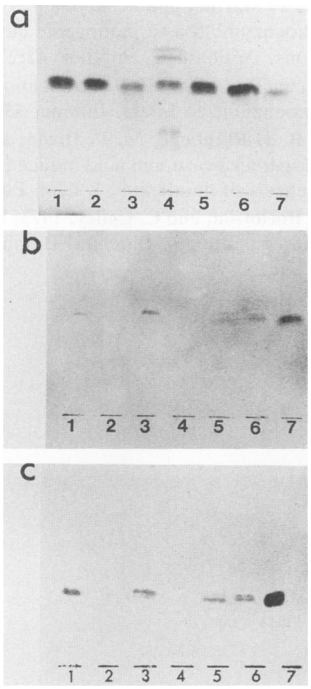

FIG. 4. Comparison of binding to PEs of various sources. Samples of PE (5 μg) from bovine brain (lane 1), bovine liver (lane 2), porcine liver (lane 3), dog brain (lane 4), egg yolk (lane 5), soybean (lane 6), and *E. coli* (lane 7) were run on TLC and tested for binding by overlay. (a) Visualized by iodine vapor; (b) exoenzyme S binding; (c) *H. pylori* binding.

pathogenesis. It has been proposed that PE metabolism plays a role in signal transduction (8, 9). The low level of phosphatidylinositide binding might also interfere with the phosphoinositol pathway of signal transduction (16). It may also be relevant to the ADP-ribosyltransferase activity of exoenzyme S that protein synthesis elongation factor 1α is posttranslationally modified with ethanolamine phosphoglycerol (23, 26, 28). This might permit the intracellular targetting of this factor.

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