

## Binding of *Helicobacter pylori* to Sialic Acid-Containing Glycolipids of Various Origins Separated on Thin-Layer Chromatograms†

HALINA MILLER-PODRAZA,\* MAAN ABUL MILH, SUSANN TENEBERG,  
AND KARL-ANDERS KARLSSON

Department of Medical Biochemistry, Göteborg University, S-413 90 Göteborg, Sweden

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**Two standard strains of *Helicobacter pylori*, grown on solid or in liquid medium, were studied for their binding to sialic acid-containing glycosphingolipids on thin-layer plates. NCTC 11637, but not strain 11638, bound to mixtures of gangliosides of various human and animal origins with similar binding patterns and also to polyglycosylceramides of human erythrocytes, leukocytes, and placenta. There was an apparent specificity for NeuAc $\alpha$ 3Gal of the neolacto series of gangliosides, since NeuAc $\alpha$ 6Gal or ganglio-series gangliosides did not bind.**

*Helicobacter pylori* is a microaerophilic human gastric bacterium connected to gastritis and peptic ulcer disease and the development of stomach cancer (13). The bacterium apparently carries two different sialic acid-dependent binding specificities, one based on recognition of NeuAc $\alpha$ 3Gal, present in many glycoconjugates, and another based on an unknown epitope associated with polyglycosylceramides (PGCs) (9, 10). Both are expressed when the bacterium is grown on agar plates while the PGC binding appears alone after growth in liquid culture. Earlier, the simple three-sugar ganglioside GM3 (see Table 1) was reported as active (1, 5, 14) or inactive (6) when tested on thin-layer chromatography (TLC) plates. Other gangliosides have not been investigated. Recently, *H. pylori* was analyzed for hemagglutination of resialylated human erythrocytes, indicating a preference for 3-linked sialic acid (4). As a first step of a more detailed characterization of the binding epitopes involved, we tested a broad spectrum of gangliosides and PGCs from various human and animal sources on TLC

plates, with two standard isolates of *H. pylori* and the two different cultivation conditions.

Total ganglioside fractions were isolated from tissues in our laboratory according to established procedures (7). Some fractions were partitioned according to the method of Folch et al. (3), as indicated in the legends to the figures. PGCs were prepared according to the peracetylation procedure as described elsewhere (11, 12). Human leukocytes were total leukocytes obtained in the buffy coat. Bacterial cultivations and overlay on silica gel TLC plates with  $^{35}\text{S}$ -labeled *H. pylori* were performed as described previously (9).

Figure 1 shows binding of *H. pylori* to ganglioside mixtures prepared from different human tissues. The autoradiograms provide a comparison of the two strains, NCTC 11637 and NCTC 11638, shown previously (8, 10) to agglutinate erythrocytes in a sialic acid-dependent and -independent way, respectively. There was a strong binding of NCTC 11637 to gangliosides. The patterns of binding were similar for different tissues

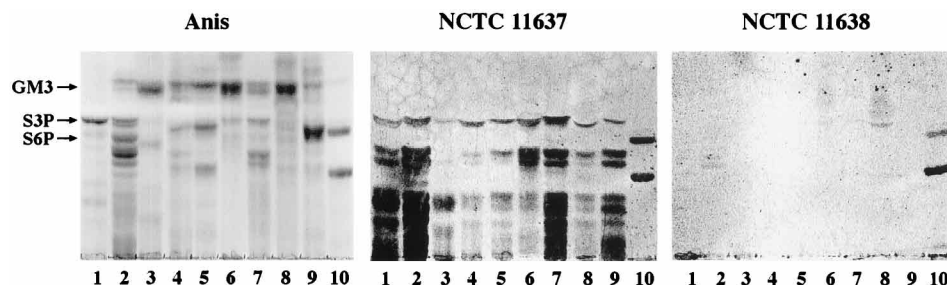


FIG. 1. Binding of *H. pylori* to gangliosides of different human tissues. Anis, plate (silica gel 60 aluminum plate; Merck, Darmstadt, Germany) sprayed with anisaldehyde (4-methoxybenzaldehyde); NCTC 11637, plate overlaid with radiolabeled  $^{35}\text{S}$ -NCTC 11637; NCTC 11638, plate overlaid with radiolabeled NCTC 11638. Lanes 1, upper-phase (after Folch's partition) gangliosides from human erythrocyte membranes; lanes 2, upper-phase gangliosides from human leukocytes; lanes 3, total gangliosides from human meconium; lanes 4, total gangliosides from human stomach; lanes 5, total gangliosides from human colon; lanes 6, total gangliosides from liver cancer; lanes 7, upper-phase gangliosides from kidney cancer; lanes 8, total gangliosides from human pancreas; lanes 9, total gangliosides from human small intestine; lanes 10, *H. pylori*-positive glycolipids from rabbit thymus. At the left, the migration of reference gangliosides is indicated. S3P and S6P stand for sialyl-3-paragloboside and sialyl-6-paragloboside, respectively. The plates were developed in chloroform-methanol-0.25% KCl in water, 50:40:10 (vol/vol/vol), respectively. Sulfatides migrate in this chromatographic system close to the solvent front and are not shown in the picture. The bacteria were grown on brucella agar plates.

\* Corresponding author. Mailing address: Department of Medical Biochemistry, Göteborg University, Medicinargatan 9A, S-413 90 Göteborg, Sweden. Phone: 46 31 773 3154. Fax: 46 31 41 31 90. E-mail: Halina.Miller-Podraza@medkem.gu.se.

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TABLE 1. Binding of *H. pylori* to purified gangliosides<sup>a</sup>

Compound	Binding
NeuAc $\alpha$ 3Gal $\beta$ 4Glc $\beta$ Cer (GM3)	-
Gal $\beta$ 3GalNAc $\beta$ 4(NeuAc $\alpha$ 3)Gal $\beta$ 4Glc $\beta$ Cer (GM1)	-
NeuAc $\alpha$ 3Gal $\beta$ 3GalNAc $\beta$ 4(NeuAc $\alpha$ 3)Gal $\beta$ 4Glc $\beta$ Cer (GD1a)	-
Gal $\beta$ 3GalNAc $\beta$ 4(NeuAc $\alpha$ 8NeuAc $\alpha$ 3)Gal $\beta$ 4Glc $\beta$ Cer (GD1b)	-
NeuAc $\alpha$ 3Gal $\beta$ 3GalNAc $\beta$ 4(NeuAc $\alpha$ 8NeuAc $\alpha$ 3)Gal $\beta$ 4Glc $\beta$ Cer (GT1b)	-
NeuAc $\alpha$ 3Gal $\beta$ 4GlcNAc $\beta$ 3Gal $\beta$ 4Glc $\beta$ Cer (S3P [sialyl-3-paragloboside])	+
NeuAc $\alpha$ 6Gal $\beta$ 4GlcNAc $\beta$ 3Gal $\beta$ 4Glc $\beta$ Cer (S6P [sialyl-6-paragloboside])	-
NeuAc $\alpha$ 3Gal $\beta$ 4GlcNAc $\beta$ 3Gal $\beta$ 4GlcNAc $\beta$ 3Gal $\beta$ 4Glc $\beta$ Cer	+

<sup>a</sup> The carbohydrate and glycosphingolipid nomenclatures are according to the recommendations of the International Union of Pure and Applied Chemistry-International Union of Biochemistry Commission on Biochemical Nomenclature (5a-5c).

although the composition of glycolipids was different, as shown by chemical staining (Anis). A relatively stronger binding was observed for minor, slowly migrating fractions, and the most rapidly moving band was located in the interval of sialyl-3-paragloboside (Table 1). The binding disappeared after treatment of gangliosides with mild acid or neuraminidase (not shown). NCTC 11638 bound weakly to some bands, but this binding was based on epitopes located internally in the carbohydrate chains (unpublished data). This was documented for both strains with reference glycolipids in lane 10 (unpublished data). Ganglioside GM3 (Table 1) was bound by NCTC 11637 only occasionally (in Fig. 1 and 2, it is negative). This inconsistency, and previous reports describing GM3 as a positive (1, 5, 14) or negative (6) binder, may be explained by a poor exposure of this three-sugar ganglioside on the assay surface.

Figure 2 shows binding of *H. pylori* to gangliosides isolated from some animal tissues (lanes 2 to 6) in comparison with typical binding to human gangliosides (lanes 1; identical to lanes 1 of Fig. 1). Animal bands correspond to bands of the human preparations. NCTC 11638 was negative for all lanes, except for some bands of lane 5 (monkey small intestine) and lane 6 (chicken erythrocyte membranes), which were unreactive with 11637. The basis of this apparently sialic acid-independent binding is not known. A strong, sialic acid-independent binding by this strain was also observed for PGC fractions isolated from monkey intestine (not shown).

The binding of *H. pylori* to some purified gangliosides is

listed in Table 1. Sialyl-3-paragloboside and its seven-sugar counterpart with neolacto core chain were positive on TLC plates, while sialyl-6-paragloboside was inactive when assayed under the same conditions. Brain gangliosides were typically negative, as was the case also for ganglioside GM3, as discussed above. A reproducible binding by NCTC 11637 was observed when the bacterium was grown on agar plates. Growth in liquid medium resulted in a weak, occasional binding.

Binding of *H. pylori* to PGCs of different sources is shown in Fig. 3. In accordance with previous reports (9, 10), the PGCs of human erythrocytes, leukocytes, and placenta (lanes 6 to 8) were strongly bound by NCTC 11637, both when grown in broth and when grown on agar. This binding was absent from plates overlaid with NCTC 11638. However, this strain bound strongly to PGC fractions prepared from rat small intestine and wild boar intestine (lanes 1 and 2) and monkey intestine (not shown). This binding did not disappear after neuraminidase treatment of glycolipids, although there were some changes in glycolipid migration on TLC plates (unpublished data). PGCs from other animal sources were negative or only slightly positive for NCTC 11637 grown on agar, which is probably based on the NeuAc $\alpha$ 3Gal epitope.

The purpose of the present work was to investigate in an overview way the two sialic acid-dependent binding specificities with a broad spectrum of gangliosides and PGCs. The results support the previously published conclusion of a NeuAc $\alpha$ 3Gal-containing epitope being recognized after growth on agar (2). This is a common epitope present in glycolipids of different human and animal tissues (Fig. 1 and 2). NeuAc $\alpha$ 3 linked to *N*-acetylglucosamine is a feature of many glycoproteins, including fetuin, which is known to inhibit hemagglutination by *H. pylori* (2, 8, 10). Concerning gangliosides, we found a relatively stronger binding to more complex species, possibly with Fuc branches. For human leukocytes (Fig. 1, lanes 2), a series of gangliosides has been structurally characterized (15, 16) as having terminal NeuAc $\alpha$ 3 and straight-chain *N*-acetylglucosamines with varying degrees of Fuc branches (Fuc $\alpha$ 3GlcNAc). The second sialic acid-dependent binding activity is linked to PGCs, is expressed alone from growth in broth (9, 10), and is apparently specific for human tissues. The broth-grown NCTC 11637 bound only to human PGCs (Fig. 3, F12 11637), although animal PGCs also contained sialic acid. Regarding NCTC 11638, the results support the idea that this strain is unable to bind to sialylated glycoconjugates but has instead an unidentified specificity not based on sialic acid.

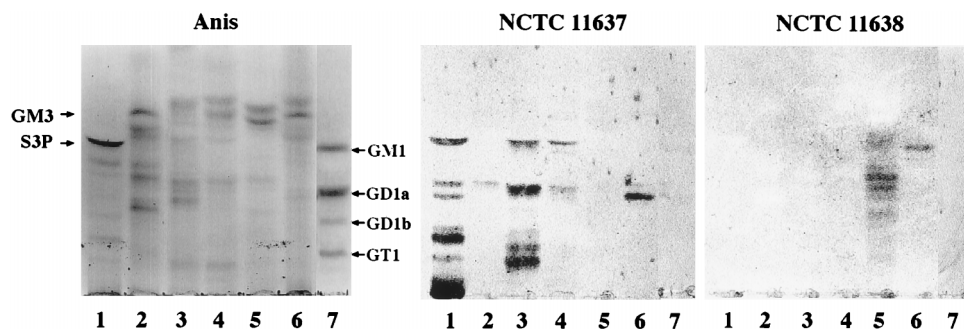


FIG. 2. Binding of *H. pylori* to gangliosides of animal tissues. Anis, plate sprayed with anisaldehyde (4-methoxybenzaldehyde); NCTC 11637, plate overlaid with radiolabeled <sup>35</sup>S-NCTC 11637; NCTC 11638, plate overlaid with radiolabeled NCTC 11638. Lanes 1, upper-phase (after Folch's partition) gangliosides of human erythrocyte membranes; lanes 2, upper-phase gangliosides of horse kidney; lanes 3, total gangliosides of rabbit small intestine; lanes 4, total gangliosides of guinea pig small intestine; lanes 5, total gangliosides of monkey small intestine; lanes 6, total gangliosides of chicken erythrocyte membranes; lanes 7, reference bovine brain gangliosides (Calbiochem, La Jolla, Calif.). The chromatographic conditions and abbreviations are as described for Fig. 1. Sulfatides are not shown in the picture. The bacteria were grown on brucella agar plates.

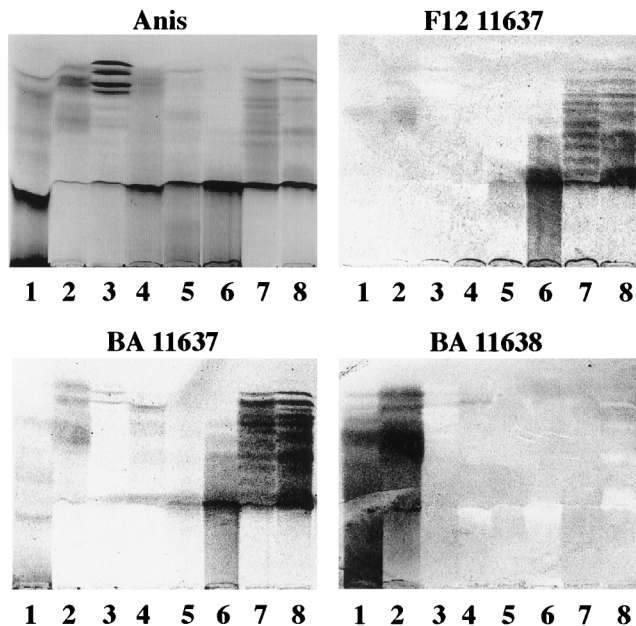


FIG. 3. Binding of radiolabeled *H. pylori* to PGC fractions of different human and animal tissues. Anis, plate sprayed with anisaldehyde (4-methoxybenzaldehyde); F12 11637, plate overlaid with NCTC 11637 grown in Ham's F-12 liquid medium; BA 11637, plate overlaid with NCTC 11637 grown on brucella agar plates; BA 11638, plate overlaid with NCTC 11638 grown on brucella agar plates. The PGCs were obtained from rat small intestine mucosa (lanes 1), wild boar small intestine mucosa (lanes 2), bovine erythrocyte membranes (lanes 3), rabbit small intestine (lanes 4), dog small intestine mucosa (lanes 5), human erythrocyte membranes (lanes 6), human leukocytes (lanes 7), and human placenta (lanes 8). The plates were developed in chloroform-methanol-water, 50:55:19 (vol/vol/vol), respectively.

The relevance of the present results of *H. pylori* binding to target cells in vivo remains unclear. It is evident that whole-tissue human stomach contains a series of receptor-active gangliosides (Fig. 1, lanes 4); however, the contribution by the mucosal part is still unknown. A strong binding to gangliosides and PGCs was observed for human leukocytes, where the dominating subpopulation, the neutrophils, is the important inflammatory cell group of the gastritis process. In separate communications, we will describe stomach and leukocyte glycoconjugates, which are being recognized by *H. pylori* in a sialic acid-dependent way.

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