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NOTES

Binding of *Actinomyces naeslundii* to Glycosphingolipids

MICHAEL J. BRENNAN,* RICHARD A. JORALMON, JOHN O. CISAR, AND ANN L. SANDBERG

Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20892

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The type 2 fimbrial lectin of *Actinomyces naeslundii* WVU45 mediated the binding of this bacterium to glycosphingolipids chromatographed on thin-layer silica gel plates. Radioiodinated bacteria attached to G_{M1}, G_{D1b}, and globoside. After chromatograms were treated with sialidase, the bacteria also bound to G_{D1a} and G_{T1b}. The actinomyces lectin apparently recognized the Galβ3GalNAc termini of these gangliosides and the GalNAcβ3Gal terminus of globoside, suggesting that glycolipids containing these sequences may serve as receptors for *A. naeslundii* on mammalian cells.

The D-galactose (Gal)-, *N*-acetyl-D-galactosamine (GalNAc)-reactive lectin associated with the type 2 fimbriae of *Actinomyces naeslundii* WVU45 and *A. viscosus* T14V mediates the interaction of these gram-positive oral bacteria with other bacteria (5, 11) and with sialidase-treated human erythrocytes (7), epithelial cells (3), and polymorphonuclear leukocytes (12). The most potent inhibitor of actinomyces lectin-mediated interactions is β-D-galactopyranosyl-(1→3)-*N*-acetyl-D-galactosamine (Galβ3GalNAc) (4, 11). Peanut agglutinin, a plant lectin that specifically recognizes this disaccharide, inhibits the attachment of *A. naeslundii* to monolayers of human oral epithelial cells (3) and recognizes a 160-kilodalton cell surface glycoprotein that apparently serves as a receptor for this bacterium on epithelial cells (2). Since certain glycosphingolipids contain the carbohydrate sequence Galβ3GalNAc, the actinomyces fimbrial lectin may also interact with mammalian cell surface glycolipids. Therefore, the binding of viable actinomyces to a number of defined glycolipids has been examined by a procedure previously utilized to detect receptors for viruses (8), bacteria (1), and antibodies (9, 10).

The gangliosides asialo G_{M2} (a gift from William Young, Jr., University of Virginia, Charlottesville), G_{M2} (Bachem, Inc., Torrance, Calif.), asialo G_{M1} (Supelco, Inc., Bellefonte, Pa.), and G_{M1} (Supelco) were chromatographed on high-performance thin-layer chromatography silica gel plates (article no. 5547; Camag Scientific, Inc., Wrightsville Beach, N.C.) with chloroform-methanol-water (50:40:10, vol/vol) for 45 min and detected with orcinol reagent (Fig. 1a). Attachment of radioiodinated bacteria to the gangliosides was assessed on a duplicate chromatogram. The chromatogram was dipped in polyisobutylmethacrylate (Polysciences Inc., Warrington, Pa.) for 1 min and blocked with 0.5% bovine serum albumin in phosphate-buffered saline (0.02 M PO_4^{3-}, 0.15 M NaCl [pH 7.2]) for 2 h. *A. naeslundii* WVU45 was cultured as previously described (7); washed in Hanks balanced salt solution (Flow Laboratories, Inc., McLean, Va.) containing 0.2 mg of $CaCl_2$ per ml, 0.2 mg of $MgSO_4$ per ml, and 0.2% bovine serum albumin (HBSS$^+$); adjusted to 10^9 cells per ml; and incubated for 1 h at 22°C with 25 μg of R64 rabbit immunoglobulin G per ml that is reactive with nonfimbrial bacterial surface antigens (6). The bacteria were washed with HBSS$^+$, incubated with 10^6 cpm of ^{125}I-protein A (Amersham Corp., Arlington Heights, Ill.) for 1 h at 22°C, washed thoroughly, and resuspended in HBSS$^+$. The radiolabeled bacteria retained lectin activity as determined by hemagglutination and epithelial cell adherence assays (3, 7). Radioiodinated actinomyces (5×10^8 cells, 4×10^5 cpm) were layered on chromatograms, and the plates were incubated for 90 min at 25°C and washed three times with HBSS$^+$. The chromatograms were air-dried, and attached bacteria were detected by autoradiography with XAR-5 film (Eastman Kodak, Rochester, N.Y.) and Cronex Quanta III intensifying screens (Du Pont Co. Wilmington, Del.) at −70°C.

A. naeslundii bound to asialo G_{M1} and G_{M1}, which have terminal Galβ3GalNAc (Fig. 1b, lanes 7 and 8), but did not bind to asialo G_{M2} or G_{M2} (Fig. 1b, lanes 5 and 6), the immediate biosynthetic precursor to G_{M1} which lacks only the β-linked terminal D-galactose (Table 1). A mixture of brain gangliosides containing G_{M1}, G_{D1a}, G_{D1b}, and G_{T1b} (Sigma Chemical Co., St. Louis, Mo.) was also separated by thin-layer chromatography and detected by orcinol staining

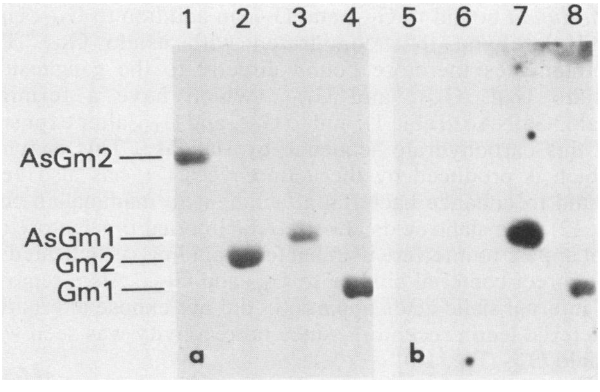

FIG. 1. Binding of *A. naeslundii* WVU45 to thin-layer chromatograms of gangliosides. Duplicate chromatograms were (a) stained with orcinol or (b) incubated with radioiodinated *A. naeslundii* WVU45, and bands were detected by autoradiography, as described in the text. Approximately 3 μg of asialo G_{M2} (AsGm2; lanes 1 and 5), G_{M2} (Gm2; lanes 2 and 6), asialo G_{M1} (AsGml; lanes 3 and 7), or G_{M1}(Gml; lanes 4 and 8) was applied.

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* Corresponding author.

TABLE 1. Oligosaccharide structure of glycosphingolipids

Glycolipid	Oligosaccharide structure
Ganglioside[a]	
G_{M2}	GalNAcβ1-4Galβ1-4Glcβ1→ | α2,3 NeuAc
G_{M1}	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1→ | α2,3 NeuAc
G_{D1a}	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1→ | α2,3 | α2,3 NeuAc NeuAc
G_{D1b}	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1→ | α2,3 NeuAc | α2,8 NeuAc
G_{T1b}	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1→ | α2,3 | α2,3 NeuAc NeuAc | α2,8 NeuAc
Neutralglycolipid	
CTH	Galα1-4Galβ1-4Glcβ1→
Globoside	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1→
Forssman glycolipid	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1→

[a] Abbreviated according to Svennerholm (13).

(Fig. 2, lane 1). *A. naeslundii* bound directly to G_{M1} and G_{D1b} and also to a band that was not routinely detected by orcinol staining but comigrated with asialo G_{M1} (Fig. 2, lane 2). Recognition of this latter band by the bacterium overlay suggests that this technique is more sensitive for detection of glycolipids than is chemical staining, as previously indicated (1, 8). An identical chromatogram was treated with 3×10^{-2} U of sialidase (type X from *Clostridium perfringens*; Sigma) per ml in 0.05 M sodium acetate [pH 5.5] containing 0.01 M $CaCl_2$ for 2 h at 37°C (Fig. 2, lane 3). After this treatment, *A. naeslundii* bound to G_{D1a} and G_{T1b} in addition to G_{M1}, G_{D1b} and the band that comigrated with asialo G_{M1}. The actinomyces therefore bound directly to the gangliosides asialo G_{M1}, G_{M1}, and G_{D1b}, which have a terminal Galβ3GalNAc (Table 1), and to G_{D1a} and G_{T1b} after exposure of this carbohydrate sequence by sialidase. This enzyme, which is produced by the actinomyces (7), has also been found to enhance bacterial attachment to mammalian cells (3, 12). The sialic acids linked to the internal D-galactose did not appear to interfere with bacterial binding, as indicated by the direct bacterial binding to G_{M1} and G_{D1b}. Also, removal of internal sialic acids apparently did not expose alternative bacterial lectin receptors, since no reactivity was seen with asialo G_{M2} (Fig. 1b).

The lectin associated with the type 2 fimbriae mediates the binding of *A. naeslundii* to the gangliosides. The actinomyces did not bind to these glycolipids in the presence of 0.1 M lactose (Fig. 2, lane 4), and a radiolabeled mutant strain of *A. naeslundii* WVU45, WVU45M, that specifically lacks the type 2 fimbriae (6) did not bind to any of the gangliosides (Fig. 2, lane 5).

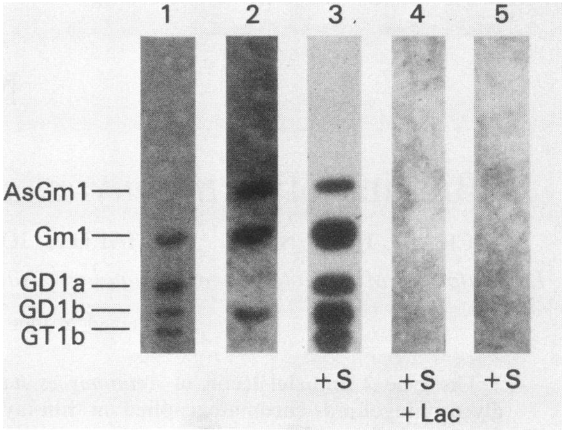

FIG. 2. Binding of actinomyces to mixed brain gangliosides separated by thin-layer chromatography. Approximately 3 μg of gangliosides was applied to each lane, and chromatograms were stained with orcinol (lane 1), incubated with radioiodinated *A. naeslundii* WVU45 (lanes 2, 3, and 4), or incubated with radioiodinated *A. naeslundii* WVU45M, a mutant specifically lacking type 2 fimbriae (lane 5). Bands on chromatograms incubated with bacteria were detected by autoradiography as described in the text. Some of the chromatograms were treated with sialidase (+S) before the addition of bacteria (lanes 3, 4, and 5), and one of these (lane 4) was incubated with bacteria in the presence of 0.1 M lactose (+Lac). Abbreviations: AsGml, asialo G_{M1}; Gml, G_{M1}; GDla, G_{D1a}; GDlb, G_{D1b}; GTlb, G_{T1b}.

The globo-series glycosphingolipids globotriaosylceramide (CTH; Supelco), globotetraosylceramide (globoside; Supelco), and IV^3-*N*-acetylgalactosaminyl-β-globotetraosylceramide (Forssman glycolipid; a gift from William Young, Jr., University of Virginia, Charlottesville) were also chromatographed, stained with orcinol (Fig. 3a), and overlaid with radioiodinated *A. naeslundii* WVU45 (Fig. 3b). The oligosaccharide structures of these glycolipids (Table 1) contain the P blood group determinant Galα4Gal, which is a receptor for the uropathogenic *Escherichia coli* (1). This sequence is not recognized by the actinomyces lectin, since

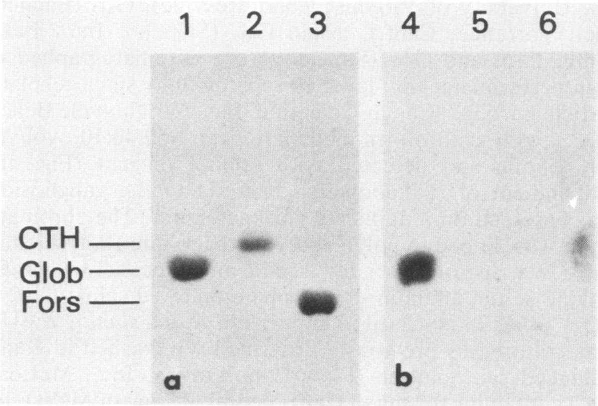

FIG. 3. Binding of *A. naeslundii* WVU45 to thin-layer chromatograms of neutral glycosphingolipids. Duplicate chromatograms were (a) stained with orcinol or (b) incubated with radioiodinated *A. naeslundii* WVU45, and bands were detected by autoradiography, as described in the text. Approximately 3 μg of globoside (Glob; lanes 1 and 4), CTH (lanes 2 and 5), or Forssman glycolipid (Fors; lanes 3 and 6) was applied.

A. naeslundii bound to globoside (Fig. 3b, lane 4) but not to CTH (Fig. 3b, lane 5). The terminal GalNAcβ3Gal of globoside was specifically recognized by the actinomyces lectin, whereas the terminal GalNAcβ3GalNAc of the Forssman glycolipid (Fig. 3b, lane 6) and the terminal GalNAcβ4-Gal of asialo G_{M2} (Fig. 1b) were not. Bacterial attachment to globoside was inhibited by 0.1 M lactose, and the *A. naeslundii* mutant specifically lacking the type 2 fimbriae did not bind to this glycolipid (data not shown).

The direct binding of actinomyces to glycolipids on thin-layer chromatograms has significantly extended our knowledge of the reactivity of the actinomyces lectin. Although the physiological relevance of glycolipids as cell surface receptors for the actinomyces remains to be determined, these studies demonstrate that actinomyces can bind to specific glycosphingolipids and suggest that these molecules should be considered potential receptors on eucaryotic cell surfaces.

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