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Specific gangliosides function as host cell receptors for Sendai virus

(paramyxovirus/N-acetylneuraminic acid/sialoglycoconjugate/cell membrane glycolipids/Madin–Darby bovine kidney epithelial cells)

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ABSTRACT **The ability of specific gangliosides to function as host cell receptors for Sendai virus was investigated by using Madin–Darby bovine kidney cells which become resistant to infection upon treatment with *Vibrio cholerae* sialidase. Sialidase-treated cells were incubated for 20 min at 37°C with individual, highly purified gangliosides containing homogeneous carbohydrate moieties and then inoculated with virus for 10 min. Susceptibility of the cells to infection was monitored by hemagglutination titer of the virus produced 48 hr after inoculation. Incubation of the cells with gangliosides containing the sequence NeuAcα2,3Galβ1,3GalNAc (i.e., GD1a, GT1b, and GQ1b) fully restored susceptibility to infection to the cells. However, the ganglioside GQ1b in which the sequence ends with two sialic acids in a NeuAcα2,8NeuAc linkage instead of a single sialic acid as in GD1a and GT1b, was effective as a receptor at a concentration 1/100th that of any of the other gangliosides tested. Incubation with gangliosides similar in structure to GD1a, GT1b, and GQ1b but lacking the sialic acid attached to the terminal galactose (i.e., GM1 and GD1b) had no effect. The results from control experiments in which gangliosides were incubated at 0°C with cells or in which trypsin was used to remove gangliosides adsorbed to cells were consistent with the premise that the gangliosides must actually insert into the cellular membrane to function as Sendai virus receptors. Addition of 4×10^6 molecules of ^{14}C-labeled GD1a per cell made the cells fully susceptible to infection. Analysis of the ganglioside content of cell membranes showed that gangliosides GD1a, GT1b, and GQ1b are natural components of these cells and are present in quantities sufficient to act as receptors. These results demonstrate that gangliosides with the proper carbohydrate sequence, such as GD1a, GT1b, and GQ1b, function as natural receptors for Sendai virus in host cells.**

During the initial phase of infection, paramyxoviruses such as Sendai virus adsorb to and fuse with surface membranes of their host cells. These early events require the presence of specific components in the host membrane which minimally serve as viral attachment sites. The molecular nature of the cell surface receptors for paramyxoviruses, in particular for Sendai and Newcastle disease viruses, has been extensively investigated (2–10). Early studies had revealed that sialic acids of cell surface oligosaccharides are an essential feature of the viral receptor determinant because sialidase treatment of certain host cells can prevent viral infection (2–4). More recent investigations have demonstrated that the specificity of Sendai virus for its host cell receptors is determined by more than just the presence of sialic acid.

Host cells made resistant to infection by Sendai virus by removal of endogenous receptors with *Vibrio cholerae* sialidase were made fully susceptible again by resialylation of the cell surface with β-galactoside α2,3-sialyltransferase but not β-galactoside α2,6-sialyltransferase (10). Based on the known specificity of these enzymes, the resialylation experiments suggest that the carbohydrate sequence NeuAcα2,3Galβ1,3GalNAc is important for the interaction of Sendai virus with host cells (7, 10). However, the results of the study by Holmgren *et al.* (9) which examined the binding of Sendai virus to monolayers of gangliosides indicated that, although this sequence did bind the virus, the higher affinity exhibited by gangliosides containing the sequence NeuAcα2,8NeuAcα2,3Galβ1,3GalNAc made it a more likely candidate for the recognition-specific structure of the receptor for Sendai virus.

Both oligosaccharide sequences are found on cell surface sialoglycolipids (gangliosides) and possibly on sialoglycoproteins. The ability of either type of sialoglycoconjugate, when incorporated into artificial membranes (5, 6, 8, 11), to adsorb paramyxoviruses and to compete with erythrocytes for virus as measured by hemagglutination (HA) inhibition suggests that both types may act as host cell receptors. However, the actual functioning of cell surface gangliosides or sialoglycoproteins during infection has not been previously described.

In the study reported here, gangliosides were investigated to determine if they act as host cell receptors for Sendai virus during the adsorption-fusion phase of viral infection. A preliminary report of some of these results has been presented (12).

MATERIALS AND METHODS

Virus and Cells. Parainfluenza 1 virus [Sendai virus, Z strain; also known as hemagglutinating virus of Japan (HVJ)] was propagated in 11-day embryonated chicken eggs and purified under sterile condition by using the vertical rotor centrifugation method (13). The purified virus was stored at −70°C until used. Madin–Darby bovine kidney (MDBK) cells were grown in reinforced Eagle's medium (RE medium) (14) containing 10% fetal calf serum and used for experiments when the monolayer culture reached 90–100% confluency (2×10^6 cells per 35-mm dish). Cells were counted by using a hemocytometer after trypsinization.

Preparation and Characterization of Gangliosides. Gangliosides were isolated from human brain (15) and separated according to their number of sialic acids on a Spherosil-DEAE-dextran column prepared as described (16). Mono-, di-, tri-, and tetrasialogangliosides were eluted in separate fractions from the column with a discontinuous gradient of potassium acetate in

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Abbreviations: MDBK cells, Madin–Darby bovine kidney cells, RE medium, reinforced Eagle's medium; HA, hemagglutination; HAU, hemagglutination unit. Abbreviations for gangliosides follow the nomenclature system of Svennerholm (1).

methanol. Individual gangliosides in the fractions were isolated by chromatography on silica gel columns or thin-layer plates.

The purity of individual gangliosides was assayed by analyses of fatty acids and sphingosine moieties after acid hydrolysis (17) and of the carbohydrate moieties as alditol acetates after trifluoroacetolysis (18) and by mild partial acid hydrolysis and stepwise hydrolyses with specific exoglycosidases, permethylation, and mass spectrometric determination of intact gangliosides and hydrolysis products (18). Individual gangliosides were found to be at least 99% homogeneous with regard to their carbohydrate composition by the above criteria.

Possible protein contamination of the purified gangliosides was assessed by radioiodination. A 50-μg aliquot of each ganglioside was dissolved in Tris/saline buffer (0.14 M NaCl/0.7 mM Na phosphate/0.025 M Tris adjusted to pH 7.2 with HCl) containing 2% $NaDodSO_4$ and iodinated by reaction with 10 μg of Iodo-gen (Pierce) and 100 μCi (1 Ci = 3.7×10^{10} becquerels) of $Na^{125}I$ (Amersham) for 15 min at 21°C as described for viral proteins (9). Carrier iodide and 2-mercaptoethanol were added to the iodinated samples to final concentrations of 0.25 M and 5%, respectively. The ganglioside samples were electrophoresed on 5–12.5% polyacrylamide gradient gels (19) with protein molecular weight standards that had been iodinated under the same conditions. By comparing the amount of radioactivity retained in the processed gel (13) to that of protein standards it was estimated that <0.02% of the dry weight of the purified gangliosides was protein. Commercial preparations (Suppelco) of GM1, GD1, and GT1 were analyzed in the same manner and found to contain considerably greater amounts of protein (about 5% of the dry weight) which were visible even on Coomassie blue-stained gels.

^{14}C-Labeled GD1a was prepared enzymatically by incubating 15 nmol of GM1 and 54 nmol of CMP-[^{14}C]N-acetylneuraminic acid (276 mCi/mmol, New England Nuclear) with 1.1 milliunits of β-galactoside α2,3-sialyltransferase in 100 μl of 0.05 M NaCl/0.05 M sodium cacodylate, pH 6.5. After 20 hr at 37°C, the reaction mixture was diluted with chloroform and methanol to a final chloroform/methanol/water ratio of 60:30:4.5 (vol/vol) and applied to a 1-g column of Sephadex G-25 (superfine) equilibrated in the same solvent mixture. The column was eluted with 7 ml of the same solvent. Analysis of the product by thin-layer chromatography revealed virtually quantitative conversion of GM1 to GD1a and that at least 97% of the ^{14}C-labeled product comigrated with authentic GD1a.

To analyze the ganglioside content of MDBK cells, a cell membrane fraction was prepared (13) from 1×10^8 cells scraped from 15 confluent 10-cm dishes. The gangliosides extracted from this fraction were analyzed as described in this section. The recovery of total sialic acid in the membrane fraction was 20%.

Cellular Treatments. Before treatment, MDBK cell monolayers in 35-mm dishes were washed once with Tris/saline. Endogenous cellular receptors for Sendai virus were destroyed by incubation of the cells with 25 units of *V. cholerae* sialidase (neuraminidase, 500 units/ml, GIBCO) for 2 hr at 37°C in 0.50 ml of Tris/saline/albumin (Tris/saline containing 10 mg of bovine serum albumin per ml). The cells thus treated are hereafter referred to as "sialidase-treated cells." The sialidase-treated cells were washed twice with 1 ml of Tris/saline. The amount of sialic acid released from the cells by treatment with sialidase was determined by the thiobarbituric acid procedure (20).

Individual, purified gangliosides were dispersed in 0.5 ml of RE medium by vigorous vortexing for 2 min and then incubated with sialidase-treated cells for 20 min at 37°C. Unadsorbed ganglioside was removed by washing the cells twice with 1 ml of Tris/saline/albumin. Cells were then inoculated with virus to assess their susceptibility to infection.

As standard procedure for inoculation of monolayers, cells were overlaid with 0.25 ml of Tris/saline containing Sendai virus at a multiplicity of infection of 3 infectious particles per cell and were incubated for 10 min at 37°C. After three washes with Tris/saline, 2 ml of growth medium was added to each dish and the cells were incubated for 48 hr at 37°C. Virus production was measured by HA titer of the infected culture medium with formalinized chicken erythrocytes (21). Inoculation conditions were selected such that production of progeny virus was proportional to the amount of inoculum.

To quantitate ganglioside incorporation, ^{14}C-labeled GD1a (1.2 μg) was added to sialidase-treated cells by the same procedure described for unlabeled gangliosides. After the two Tris/saline/albumin washes, adsorbed [^{14}C]GD1a was removed from cells by treatment of the monolayers with 0.05% trypsin (1:250, Difco) in 0.8% NaCl/0.04% KCl/0.1% glucose/0.058% $NaHCO_3$/0.02% EDTA for 20 min at 37°C. This treatment removed >99% of the cells from the dish. Growth medium (1.5 ml) was added to the suspended cells and they were centrifuged at 1000 × g for 10 min. The resuspended pellet was inoculated with virus and the cells were replated in growth medium. Aliquots of each fraction were assayed for ^{14}C-labeled GD1a by liquid scintillation spectrophotometry.

RESULTS

Restoration of Susceptibility to Infection by GD1a. Native MDBK cells routinely produced a HA titer of 256 hemagglutination units (HAU) per ml of infected culture medium at 48 hr after inoculation (Table 1). As reported (10), treatment of these cells with *V. cholerae* sialidase for 2 hr made them resistant to infection by Sendai virus as evidenced by the lack of detectable virus production (<2 HAU/ml). This treatment removed 15 nmol of sialic acid per 35-mm dish of MDBK cells. Exogenously added GD1a which contains the sequence NeuAcα2,3Galβ1,3GalNAc restored the susceptibility of the cells to infection. The incubation time with gangliosides was limited to 20 min, a period in which endogenous restoration of receptors by the sialidase-treated cells is neglible (10).

The degree of restoration depended on the amount of ganglioside added. Largest restoration was achieved with 2.5 μg of GD1a, and the effect plateaued with larger amounts of ganglioside at a level of virus production of 64 HAU/ml (Table 1). Although values of 128 HAU/ml were obtained occasionally with 2.5 μg of GD1a, this was still lower than virus production by native cells (256 HAU/ml) inoculated with the same amount

Table 1. Restoration of susceptibility to infection by GD1a

Cell preparation	Treatment	Virus produced,* HAU/ml
Native	None	256
Sialidase-treated	None	<2
Sialidase-treated	GD1a, 25 μg	64
Sialidase-treated	2.5	64
Sialidase-treated	0.25	4
Sialidase-treated	0.025	<2
Sialidase-treated	Inoculation, then 2.5 μg of GD1a	<2

The susceptibility to Sendai virus infection of MDBK cells which had been treated with sialidase for 2 hr and then with exogenous GD1a for 20 min at 37°C before inoculation was compared to that of untreated (native) cells, sialidase-treated cells, and sialidase-treated cells incubated with GD1a after inoculation.

* Sendai virus production was measured by HA titer of the infected culture medium 48 hr after inoculation.

of virus. A possible explanation for this was suggested by the appearance of the ganglioside-treated monolayers immediately after the last wash to remove unadsorbed virus—i.e., there seemed to be fewer cells. Cell counts of the monolayers revealed that an average of 56% of the ganglioside-treated cells had become detached from the dish. No significant cell loss was observed from inoculated monolayers of native or sialidase-treated cells or native cells incubated with gangliosides. Only the sequential combination of treatments with sialidase, gangliosides, and virus resulted in cell loss. The amount of virus (64 HAU/ml) produced by the 44% of the ganglioside-treated cells that remained attached to the dish was the same as that produced by that number of native cells. This indicated that the ganglioside-treated cells were fully susceptible to Sendai virus infection.

Incubation of sialidase-treated cells with GD1a after inoculation produced no detectable restoration of susceptibility to infection (Table 1). The same result was obtained when the ganglioside was added simultaneously with the virus or allowed to incubate with the virus 20 min before inoculation of sialidase-treated cells (data not shown). This pinpointed the effect of the gangliosides to the adsorption-fusion stage of viral infection—i.e., at the cell surface as virus receptors.

Evidence for the Incorporation of Exogenous Gangliosides. The conditions we used for the incubation of exogenous gangliosides with sialidase-treated cells were based on those described for GM1 uptake in cholera toxin receptor studies (22–25). It has been shown that exogenous GM1 can spontaneously insert into the membrane of intact cells (23–24). However, a study of ganglioside uptake indicated that 70–85% of cell-associated gangliosides are merely adhering to cell surface proteins and are liberated by mild trypsin treatment (25).

A series of experiments was designed to test if the GD1a had to be incorporated into the cell surface membrane for it to function as a receptor for Sendai virus. The first set of experiments was based on the assumption that gangliosides will adsorb to cells at 0–4°C as reported (23) but will not insert into the lipid bilayer until the membrane becomes fluid at a higher temperature. Incubation of sialidase-treated cells with GD1a at 0°C instead of 37°C produced no restoration of susceptibility to infection (<2 HAU/ml) (Table 2). Incubation at 0°C did not impair the capacity of the sialidase-treated cells to produce Sendai virus. When the cells were shifted to a higher temperature (37°C) and the incubation with GD1a was continued, the same amount of virus was produced (64 HAU/ml) as by cells that had not undergone the cold treatment.

In the second set of experiments it was assumed that gangliosides that were merely adsorbed onto cell surface proteins could be removed by mild treatment of the cell surface with trypsin but those actually integrated into the lipid bilayer would not be affected. By using 1.2 μg of ^{14}C-labeled GD1a it was observed that 91% of the ganglioside added to sialidase-treated cells was removed by aspiration and the two Tris/saline/albumin washes. When the cells were then trypsinized off their dish and washed again, 4% of the ganglioside remained associated with the dish, 3% was removed by the wash, and only 2% of the total added ganglioside remained associated with the cells in suspension. Yet, when these trypsinized cells were inoculated with virus, they produced the same amount of virus (64 HAU/ml) as sialidase-treated cells that were incubated with GD1a but had not undergone the trypsin treatment (Table 2). Trypsinization of native and sialidase-treated cells did not change their susceptibility to infection (data not shown). The 2% of the added ganglioside (about 13 pmol per dish of 2×10^6 cells or 4×10^6 molecules per cell) that were functioning as Sendai virus receptors were in a trypsin-insensitive state, presumably inserted into the cellular membrane. Native MDBK cells incorporated the same amount of GD1a during the 20-min incubation as did the sialidase-treated cells (data not shown).

Table 2. Evidence of incorporation of gangliosides that function as Sendai virus receptors

Cell preparation	Treatment*	Virus produced,† HAU/ml
Sialidase-treated	None	<2
Sialidase-treated	GD1a for 20 min at 37°C	64
Sialidase-treated	GD1a for 60 min at 0°C	<2
Sialidase-treated	GD1a for 60 min at 0°C, then 20 min at 37°C	64
Sialidase-treated	GD1a for 20 min at 37°C, then trypsinization	64

* Sialidase-treated cells were incubated with 2.5 μg of GD1a under the various conditions listed before inoculation with Sendai virus.
† Sendai virus production was measured by HA titer of the infected culture medium 48 hr after inoculation.

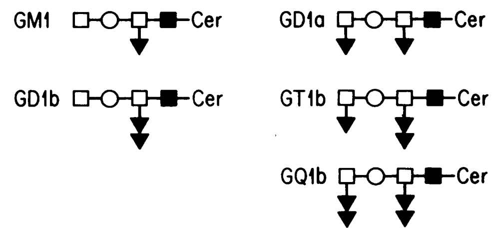

FIG. 1. Chemical structure of gangliosides. Cer, ceramide; ■, glucose; □, galactose; ○, *N*-acetylgalactosamine; ▼, *N*-acetylneuraminic acid. Gangliosides at the left contain the sequence NeuAcα,2,3Galβ1,3GalNAc and act as receptors for Sendai virus. Those at the right lack the sialic acid on the terminal galactose and do not function as receptors for Sendai virus.

Specific Gangliosides Function as Receptors for Sendai Virus. Sialidase-treated cells were incubated for 20 min with individual, highly purified gangliosides (Fig. 1) containing homogeneous carbohydrate moieties before inoculation with Sendai virus. Incubation of the cells with GD1a, GT1b, and GQ1b produced infection. All of these gangliosides contain the oligosaccharide sequence NeuAcα2,3Galβ1,3GalNAc but in

Table 3. Sendai virus infection of sialidase-treated cells after incubation with specific gangliosides

Added ganglioside type, amount in μg	Virus produced,* HAU/ml
None	<2
GM1, 2.5	<2
GD1a, 2.5	64
0.25	4
0.025	<2
GD1b, 2.5	<2
GT1b, 2.5	64
0.25	4
0.025	<2
GQ1b, 0.25	128
0.025	64
0.0025	4

The susceptibility to Sendai virus infection of sialidase-treated cells was compared to that of sialidase-treated cells incubated with various exogenous gangliosides for 20 min at 37°C.
* Sendai virus production was measured by HA titer of the infected culture medium 48 hr after inoculation.

Table 4. Lipid analysis of MDBK cells

Component	Content, nmol/mg protein
Phospholipids,	
neutral	370
acidic	81
Cholesterol	12
Protein-bound sialic acid	20
Lipid-bound sialic acid (gangliosides)	8.1
GM3	5.500
GM2	1.300
GM1	Trace
GD1a	0.200
GD1b	0.120
GT1b	0.135
GQ1b	0.020

A membrane fraction was prepared from 1×10^8 MDBK cells with a 20% recovery of total sialic acid and analyzed for lipid content. Total protein content of the sample was 5.2 mg.

GQ1b the sequence ends in the terminal linkage NeuAcα2,8NeuAc instead of single sialic acid as in GD1a and GT1b. Incubation of sialidase-treated cells with gangliosides similar in structure but lacking the sialic acid on the terminal galactose (i.e., GM1 and GD1b) produced no detectable virus (Table 3). This was consistent with our original observation that sialidase-treated cells are resistant to infection because the sialidase treatment fails to remove sialic acid residues from GM1 and will convert the endogenous GD1a, GT1b, and GQ1b to GM1 (26).

By varying the amount of ganglioside incubated with the cells it was determined that the tetrasialoganglioside GQ1b was effective as a receptor at 1/100th the concentration of any of the other gangliosides tested. Incubation of sialidase-treated cells with 2.5 μg of GM1 plus 0.025 μg of GQ1b produced the same restoration of susceptibility to infection as did incubation with 0.025 μg of GQ1b alone (data not shown). The specificity of interaction of Sendai virus was confirmed by using gangliosides GD1a and GQ1b independently prepared and generously supplied by Robert W. Ledeen.

Ganglioside Content of Native MDBK Cells. To determine if gangliosides with the proper carbohydrate sequence exist in sufficient quantities in native cells to function as receptors for Sendai virus, the ganglioside content of MDBK cells was analyzed. A membrane fraction was prepared from 1×10^8 cells. About 30% of the bound sialic acid was found in gangliosides (Table 4). About 11% of the gangliosides were of the type that have the proper oligosaccharide sequence to function as receptors for Sendai virus on these cells. About 20% of the total sialic acid was recovered in the membrane fraction. With adjustment for recovery, GD1a was found in a concentration of approximately 100 pmol per dish of 2×10^6 cells, GT1b at 70 pmol, and GQ1b at 10 pmol.

DISCUSSION

To qualify as a virus receptor, a macromolecule must not only specifically bind the virus but also promote the infectious process. In this study, gangliosides such as GD1a, GT1b, and GQ1b were shown to fulfill both requirements of the definition of a virus receptor. They were specific in their interaction with Sendai virus and conferred susceptibility to infection as integral components of the cell surface membrane. That not all species of gangliosides serve equally well as receptors for Sendai virus was first suggested by Haywood (27) who demonstrated that commercially prepared di- and trisialogangliosides inhibit hemagglutination by Sendai virus more effectively than do monosialogangliosides when they are incorporated into liposomes. In their binding studies Holmgren *et al.* (9) used individual, highly purified gangliosides adsorbed to polystyrene Petri dishes to elucidate the recognition-specific structure involved in the high-affinity binding of Sendai virus to the cell surface. Results of our current study of infection of host cells are in excellent quantitative agreement with the finding by Holmgren *et al.* (9) that gangliosides containing a terminal disialosyl linkage such as GQ1b are 100-fold more effective in serving as receptors for Sendai virus than are less complex gangliosides such as GD1a and GT1b. In addition, our results show that each of these gangliosides is sufficient to impart full susceptibility to infection to sialidase-treated cells.

The sialidase treatment that makes MDBK cells resistant to infection removed 15 nmol of sialic acid per dish of MDBK cells. The present data demonstrate that the functional integration of 13 pmol of GD1a per dish or 4×10^6 molecules per cell resulted in the same susceptible state. This number actually may overestimate the amount of GD1a needed to restore susceptibility because the mild trypsin treatment of the cell surface may not remove all of the adsorbed ganglioside. Our previous study demonstrated that the transfer of 320 pmol of sialic acid to the cell surface by purified β-galactosideα2,3-sialyltransferase restores full susceptibility to sialidase-treated cells (10). Although >95% of the NeuAc incorporated by MDBK cells appears to be associated with glycoproteins, it is not possible to exclude a small amount (5% or less) incorporated by gangliosides. This is more than sufficient to confer susceptibility to infection.

Analysis of the ganglioside content of native MDBK cells indicated that the amount of GD1a and GT1b naturally present (100 and 70 pmol per dish, respectively) is sufficient to account for the susceptibility of these cells to infection. Moreover, the cells contain substantial amounts (10 pmol per dish) of GQ1b which has 100-fold higher affinity for Sendai virus. As a cell type, MDBK cells have a relatively low ganglioside content (28). Considering then the occurrence of higher levels of gangliosides in many mammalian cell types it is not surprising that Sendai virus has been found to interact with a wide variety of cells, either as an infectious agent or as a cell fusogen.

The results of the binding study by Holmgren *et al.* (9), resialylation experiments (7, 10), and the current study indicate that the minimal structure recognized by Sendai virus is the sequence NeuAcα2,3Gal, although the related sequence NeuAcα2,8NeuAcα,2,3Gal confers maximal binding capacity. At present it is not known whether the *N*-acetylgalactosamine residue is part of the receptor determinant or provides the proper spacing in the oligosaccharide chain. Also, although it is clear that the carbohydrate portion of the ganglioside is an essential feature of the receptor determinant, these studies do not indicate whether the rest of the molecule plays a modulating role in its interaction with the virus or simply serves to anchor it in the lipid bilayer.

In addition to gangliosides, oligosaccharides of cell surface glycoproteins contain the NeuAcα2,3Galβ1,3GalNAc sequence. Studies based on enzymatic modification of the cell surface by using *V. cholerae* sialidase to destroy endogenous receptors and β-galactosideα2,3-sialyltransferase to replace them do not distinguish which type of sialoglycoconjugate functions as the receptor because these enzymes use both glycoproteins and glycolipids as substrates (22, 29, 30). If only the sialyloligosaccharide portion is involved in the interaction with Sendai virus as with lectins, then both sialoglycoproteins and gangliosides may function as receptors, provided that they contain the proper carbohydrate sequence. It has been suggested

that the virus first adsorbs to outer glycoproteins and then in turn attaches to membrane gangliosides to effect penetration (31). However, the actual involvement of cell surface glycoproteins as receptors in the infection of host cells by Sendai virus, such as shown in this report for gangliosides, has not yet been demonstrated.

Our experiments with receptor-deficient cells (sialidase-treated MDBK cells) pinpoint the effect of the gangliosides at the adsorption-fusion stage of viral infection but do not distinguish between these early events. The close correlation observed between the receptor specificities of the present studies and those of the binding study by Holmgren *et al.* (9) strongly suggests that gangliosides function as receptors during the initial adsorption of the virus to the host cell. This interaction of Sendai virus with gangliosides such as GD1a, GT1a, and GQ1b on the cell surface suggests a possible mechanism for inducing the ensuing virus–cell fusion. Polysialogangliosides such as GD1a and GT1 have been shown to induce cell–cell fusion when incubated with cells for 2–3.5 hr (32). It is possible that the attachment of a highly multivalent ligand such as Sendai virus (12) to the cell surface may temporarily stabilize a clustering of its receptors, the polysialogangliosides GD1a, GT1b, and GQ1b, in the very region of the host membrane where virus–cell fusion should occur. However, it remains to be demonstrated if the clustering in the membrane of these natural fusing agents would in itself be sufficient to induce the membrane fusion event.

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